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A QUANTITATIVE STUDY OF THE EFFECT
OF DIMETHYL SULFOXIDE ON THE
INTRACUTANEOUS ABSORPTION
OF HYDROCORTISONE

by

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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled: "A QUANTITATIVE STUDY OF THE EFFECT OF DIMETHYL SULFOXIDE ON THE INTRACUTANEOUS ABSORPTION OF HYDROCORTISONE", submitted by Donald G. Perrier, in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

The quantitative effect of dimethyl sulfoxide on the transepidermal, intracutaneous absorption of ^3H -labeled hydrocortisone has been measured using guinea pig skin. The amount of hydrocortisone absorbed intracutaneously was determined as a function of rest time and of dimethyl sulfoxide concentration.

Several concentrations of dimethyl sulfoxide were employed and were found to produce a significant increase in the intracutaneous absorption of hydrocortisone at each of several rest times studied. Dimethyl sulfoxide appeared to produce its major effect on hydrocortisone absorption within two hours after topical application. Dermal levels of hydrocortisone decreased with increasing rest times when the hydrocortisone was applied with dimethyl sulfoxide. As the concentration of dimethyl sulfoxide increased, the rate of decrease of dermal hydrocortisone levels appeared to accelerate. Results support an in vivo mechanism for the penetrant action of dimethyl sulfoxide.

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INTRODUCTION

The intracutaneous absorption of a drug from a topically applied vehicle may be considered as the penetration of a drug from the surface of the skin into the living epidermal and dermal tissues. This is opposed to percutaneous absorption which includes intracutaneous absorption plus subsequent systemic absorption whereby the drug penetrates from the living epidermal and dermal tissues into the circulatory system.

The popularity of hydrocortisone in local dermal therapy has been well established. Unfortunately in topical therapy the ratio of absorbed to applied drug is usually very low. Therefore, it is of interest from a formulation as well as an economic point of view to increase the absorption of hydrocortisone from a topical preparation. It would be desirable to obtain maximum intracutaneous absorption of hydrocortisone while at the same time keeping systemic absorption to a minimum thereby avoiding the undesirable systemic side effects of hydrocortisone.

One approach to improving intracutaneous absorption may be with dimethyl sulfoxide which has been used as a penetrant carrier in a variety of cases. However, the effect of dimethyl sulfoxide on intracutaneous absorption has not been clearly established as the majority of work on its penetrant effect has been done on its ability to enhance percutaneous absorption. If the mechanism of dimethyl sulfoxide is through a carrier effect then the increase in

percutaneous absorption caused by dimethyl sulfoxide does not necessarily mean that there will be a subsequent increase in intracutaneous absorption. This results since dimethyl sulfoxide may simply carry the hydrocortisone through the skin into the systemic circulation.

The primary problem in determining the effect of dimethyl sulfoxide on the intracutaneous absorption of hydrocortisone is to find a suitable method of measurement. Previous measurements have been performed systematically. They indicate that relatively long rest periods and high concentrations of dimethyl sulfoxide are required before significant hydrocortisone absorption occurs. Therefore, the main interest in the present work is to determine if dimethyl sulfoxide increases hydrocortisone absorption into the skin and to see if the magnitude of this increase can be detected at earlier rest times than established previously. Also, it is of interest to see if significant hydrocortisone absorption occurs with lower concentrations of dimethyl sulfoxide than have been reported to be necessary to enhance absorption. Therefore, these factors will be studied using a method which has been developed previously for measuring absorption into the skin.

LITERATURE SURVEY

A. HYDROCORTISONE

Hydrocortisone, the most potent of the naturally occurring anti-inflammatory steroids (1) originally was isolated from an adrenal extract and described as substance M by Reichstein in 1937 (2). It was later identified in 1938 as compound F by Kendall (2). In 1950 Wendler, Graber, Jones and Tishler (3) synthesized hydrocortisone from cortisone. This resulted in an increase in the supply and use of hydrocortisone in therapy.

Witten and Sulzberger (4) issued the initial report in 1952 on the therapeutic use of topical hydrocortisone. Since then the effectiveness of hydrocortisone in topical therapy for certain inflammations and selected dermatoses has been well established (4,5,6). It was suggested by Butler (7) that the effectiveness of topical steroids in the treatment of diseases of the skin seems to be related to the anti-inflammatory potency and to the rate and the route of penetration. This is conceivable since most diseases responsive to topical corticoids are primarily inflammatory diseases of the dermis (8).

INFLAMMATION

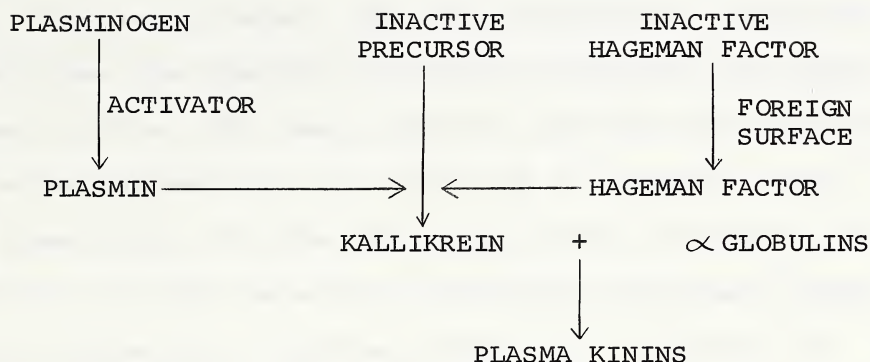
Inflammation has been described as a response to an injurious stimulus composed of a series of interrelated progressive phenomena which occur in the following order: localized cellular destruction; increased cellular perme-

ability and edema formation; stickiness of the endothelium; margination of leukocytes and, finally, entrance of non-autochthonous cells into the injured tissue (9). It has been suggested that all of the events of acute inflammation can be explained by cellular injury resulting from a lack of stabilization of the cell membrane (10). Eyring and Dougherty (11) pointed out that this localized cellular destruction results in cells primarily native to connective tissue.

The principle cell type of connective tissue is represented by the fibroblast (10,12,13). Fibroblasts are responsible for the biosynthesis of collagen (14) which is the chief supportive protein of connective tissue and the main fibrous protein of the dermis (15). As noted by Eyring and Dougherty (11) the degree of inflammation is enhanced by the autocatalytic destruction of fibroblasts. In this respect, the destruction of one cell adds to the amount of phlogogenic substance which leads to the destruction of other cells. Various phlogogenic or inflammatory producing substances have been demonstrated. Lysosomes which contain hydrolases have been linked with various processes of autolysis and necrosis (16). Weissmann (17) proposed that if an injury to a cell perforated, ruptured or in any way rendered the lysosomal membrane more permeable there may be a release of these hydrolases resulting in digestion of the parent fibroblast as well as surrounding cells. Other mediators of inflammation which

have been suggested are histamine (18,19,20), serotonin (21,22,23), bradykinin (18,23), kallikrein (24,25) and leukotaxine (26). Spector and Willoughby (22) reported that cell injury may cause activation of enzymes situated on the mast cell membrane causing lysis of the cell wall and hence the release of histamine. Several investigators (18,19,20) feel that histamine is responsible for the vasodilatation and increased vascular permeability observed after cell injury. These changes are probably the cause of the erythema and edema of inflammation (21). Increased synthesis of histamine by damaged tissue (22) or by longer acting mediators (27) may be the cause of delayed inflammation. Serotonin also was reported to be present with histamine in early inflammatory exudates and this may augment and prolong the effect of histamine in inflammation (22). Kallikrein has been shown to have the ability to increase capillary permeability (24). Leukotaxine, as well as increasing capillary permeability, has been reported to induce migration of leukocytes (26).

According to Lewis (23) plasma kinins produce vasodilatation, increased vascular permeability, pain and, in higher concentrations, may cause the accumulation and migration of leukocytes. He has suggested that these are the cardinal signs of the early stages of inflammation. Lewis (23) also has proposed a mechanism for the formation of these plasma kinins as follows:



It also has been indicated by Lewis (23) that the histamine released immediately on injury increases vascular permeability which allows an excess of fluid into the interstitial space. This reportedly causes activation of a kinin forming enzyme causing an increase in the formation of plasma kinins (23).

Cotran and Majno (28) suggest that histamine, bradykinin (a plasma kinin) and serotonin all combine to cause the blood vessels in the inflammatory foci to become abnormally permeable producing a histamine type leakage.

From the work reported it would appear that the dermis is the primary site of cutaneous inflammation.

ANTI-INFLAMMATORY MECHANISM OF HYDROCORTISONE

As has been demonstrated by the above observations, inflammation appears to be a complex sequence of events dependent upon a wide variety of factors. Perhaps, for this reason, the exact mechanism by which hydrocortisone controls inflammation has not been clearly established.

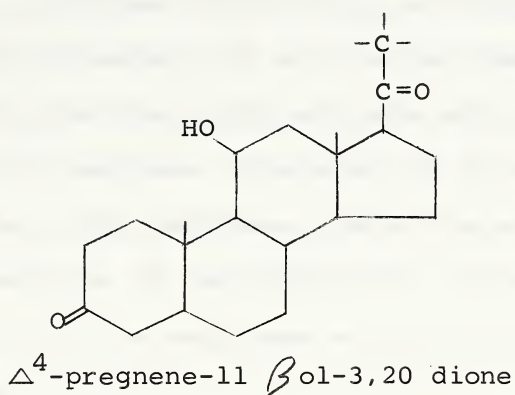
Travis and Sayers (29) have reported that hydrocortisone has the capacity to prevent or suppress the development of the local heat, redness, swelling and tenderness by which inflammation is recognized at the gross level of observation. At the microscopic level it appears to inhibit not only the early inflammatory processes (edema, fibrin deposition, capillary dilatation, migration of phagocytes into the inflamed area and phagocytic activity) but also the later manifestations (capillary proliferation, fibroblast proliferation and deposition of collagen and, still later, cicatrization) (29). Travis and Sayers (30) also noted that the anti-inflammatory effects seem to depend upon the direct local action of the steroid. The above view is supported by Dougherty and several other investigators (9,10,11) who point out that adrenocortical hormones do not inhibit the stimulus that initiates the chain reaction of inflammation. These workers contend rather that the above hormones exert their antiphlogistic effect by interrupting the chain reaction of cellular destruction that is triggered by the initiating stimulus. It has also been suggested by Travis and Sayers (30) that the anti-inflammatory property of the adrenocortical steroids derives from their effect on the metabolism of some type or types of cells participating in the process of inflammation. According to Menkin (31,32,33) hydrocortisone also exerts its anti-inflammatory activity by acting at a cellular level. It

apparently does this by impairing the activity of the injured cell so that it becomes less effective in producing some if not all of the specific chemical factors involved in inflammation.

Several groups of investigators (9,10,11) demonstrated that hydrocortisone tends to produce a rounding-up of fibroblasts thereby enhancing the resistance of surviving cells to the cytotoxic action of phlogogenic agents. Therefore, Dougherty and Eyring (11) and Dougherty and Schneebeli (9) propose that antiphlogistic hormones probably act by preventing cellular destruction by stabilizing the impermeable state of the cell membrane. Dougherty and Schneebeli (9) furthermore have pointed out that fibroblasts which survive in an area of inflammation are usually of this "rounded-up" type. They also observed that hydrocortisone concentrated within the cytoplasm of the fibroblast or at the surface of the fibroblast (10). This concentration apparently is followed by a change in the structure and metabolism of the affected cell which, in turn, is correlated with a greater resistance to the process of destruction in the inflamed area(10).

The concept that the fibroblast is the primary cell involved in inflammation and that the fibroblast is the primary target cell of hydrocortisone activity is further substantiated by the observed structure-activity relationship of anti-inflammatory steroids and their fibroblast inhibiting ability (34,35,36). The following struc-

ture has been shown to be common to all steroids that have been demonstrated to have anti-inflammatory activity in man (1).



Berliner and Ruhmann (35,36) and Berliner, Gallegos and Schneebeili (34) indicated a direct relationship between the structure of steroids, their fibroblast inhibiting potency and their ability to suppress inflammation.

Although hydrocortisone has been reported to be the most potent endogenously produced anti-inflammatory steroid (1,37) it does not produce the maximum fibroblast inhibiting activity (13). Maximum activity has been demonstrated when the hydrocortisone molecule possesses an additional double bond in A ring; 6 α , 9 α difluoro substitution and a 16, 17 acetonide structure (13) to give fluocinolone acetonide which is possibly the most potent anti-inflammatory steroid (38).

Weissmann and other investigators (39,40,41) reported that hydrocortisone acts by stabilization of lysosomal membranes. They suggest that by doing so hydrocortisone

retards the release of hydrolases from the lysosomes and thus prevents these proteolytic enzymes from destroying cells. This is in contrast with Eyring and Dougherty (11) and Dougherty and Schneebeli (9) who, as has been mentioned previously, believe that hydrocortisone acts by stabilization of the cell membrane. Therefore, it is not clear as to exactly where hydrocortisone exerts its stabilizing effect; on the membrane of the fibroblast, on the membrane of the lysosome or on the membranes of both the fibroblast and the lysosome.

Another mechanism of hydrocortisone action has been reported by Schayer (20). He indicated that the principle physiological function of glucocorticoids in inflammation would involve the vasoconstrictor effect as well as the moderation of the actions of histamine. Schayer (20) suggested that hydrocortisone by moderating the action of histamine would stabilize endothelial cells against changes due to induced histamine.

The ability of hydrocortisone to control inflammation by preventing the release of active kinin or by preventing the interaction between kallikrein and its substrate has been proposed by Cline and Melman (42).

As well as the controversy surrounding the exact mechanism of hydrocortisone in controlling inflammation, the form of hydrocortisone which is active may also be in doubt. This arises since connective tissue has been shown to metabolize hydrocortisone (38,43,44). In this respect,

Dougherty and Schneebeili (9) have indicated that none of the metabolites have any significant anti-inflammatory effect with the exception of cortisone which they observed was only 1/77 as effective as hydrocortisone.

Regardless of the form of hydrocortisone which is active or the exact mechanism by which hydrocortisone acts, it has been fairly clearly established that hydrocortisone exerts its anti-inflammatory effect in the dermal tissue.

HYDROCORTISONE ABSORPTION

The mechanism of the intracutaneous absorption of a drug may be interpreted as the process involved in effecting the penetration of a drug from the surface of the skin into the living epidermal and dermal tissues. There appears to be general agreement that the majority of drugs are absorbed by passive diffusion (45,46) and that the degree of absorption is primarily a function of the combined properties of the drug preparation and of the skin (46,47,48).

The majority of reports on drug absorption indicate that the routes by which drug molecules can be absorbed intracutaneously are through the intact epidermis (trans-epidermal) and through the skin appendages (45,46,49,50). These potential avenues have been presented by Griesemer (47).

Transepidermal absorption requires that the drug encounter the surface film and then progress through the

intact epidermis. The surface film is lipid in nature and is composed of sebum, sweat, desquamating stratum corneum and is weakly acidic in nature (51). This lipid surface film combined with the lipids in the superficial tissues of the epidermis present a lipoidal barrier. Depending on the effectiveness of such a barrier the absorption of lyophilic molecules would be enhanced through this region while the absorption of hydrophilic molecules would be resisted. It is believed that the lipids in the epidermis do play an important role in this respect (45,47,52). However, as both Rothman (53) and Malkinson (54) have pointed out, the importance of the surface film as a lipoidal barrier is probably over-emphasized. The continuous sloughing off of keratin and the presence of emulsifiers such as cholesterol in the sebum likely result in the surface film being a discontinuous film rather than an occlusive barrier.

Beneath the surface film is the stratum corneum which is composed of lipids and dead cells consisting largely of keratin, a hygroscopic, sulfhydryl-containing protein (47). The overall epidermis varies in thickness from 1/200 to 1/20 of an inch and is composed of five layers; the stratum corneum, the stratum lucidum, the stratum granulosum, the stratum spinosum and the stratum germinativum which is located immediately above the dermis (55).

It is the stratum corneum, the horny surface layer

of the epidermis, which receives the most support as being the chief barrier to intracutaneous absorption via the transepidermal route (56,57,58). Malkinson (59) demonstrated that this stratum corneum is the major barrier to the penetration of glucocorticoids. Feldmann and Maibach (60) agree that the stratum corneum is the major barrier although they also suggested that the malpighian layer (stratum spinosum) in the epidermis may act as a second barrier to the intradermal absorption of hydrocortisone. Several investigators (60,61,62,63) have postulated that, as well as acting as a barrier to hydrocortisone, the horny layer also acts as a reservoir for topically applied hydrocortisone. Malkinson (63) and Malkinson and Ferguson (61) pointed out that the excretion pattern of hydrocortisone suggests that following initial absorption a depot persists in this area which steadily releases small amounts of hydrocortisone for many days. The existence of a depot possibly accounts for the relatively slow and prolonged absorption of topically applied hydrocortisone. This thinking is supported by Feldmann and Maibach (8) who demonstrated that the absorption rate of topically applied hydrocortisone was about 0.016 per cent of the applied dose per hour with a maximum absorption rate of about 0.023 per cent per hour during the second 12 hour period after application. In another report they indicated that maximum absorption occurred on the second day when 0.080 per

cent of the applied dose was excreted as compared to 0.011 per cent on the tenth day (60). It should be noted that results reported by Feldmann and Maibach (8,60) are based on absorption of hydrocortisone through the skin. Therefore, their approach measures the amount of hydrocortisone absorbed systemically and does not indicate whether hydrocortisone absorption occurred into the dermis nor at what rate.

The proposed mechanisms by which the stratum corneum serves as a barrier also vary. Rein (64) originated the concept of the barrier being electronegative thereby attracting cations and repelling anions. Tregear (45), on the otherhand, conceived the resistance of the barrier to be a physical property of the dead, keratinized cells in the stratum corneum. The effectiveness of this barrier zone appears to vary with age and also varies over different parts of the body. For example, Feinblatt et al. (65) observed that approximately 21 per cent of topically applied hydrocortisone was absorbed through the skin of children. In contrast, other investigators (66,67,68) have shown that one per cent or less was absorbed when applied to adults. According to Feldmann and Maibach (69) hydrocortisone was absorbed to a much greater extent from the scrotum than from other areas of the body such as the palm and foot arch. These results indicate that the amount of hydrocortisone decreased as the thickness of the stratum corneum increased

with age and body location. This suggests that the stratum corneum probably acts as a physical barrier to hydrocortisone absorption.

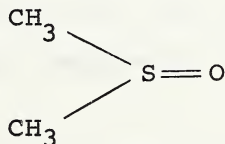
Some investigators (70,71) supported the skin appendages as avenues for penetration but other workers (72,73,74) have discounted their effectiveness in this respect. There are numerous supporters for the trans-epidermal route as the primary pathway for absorption (47,75,76,77).

From these reports it appears that the stratum corneum is the major barrier to transepidermal absorption and that this barrier is probably physical in nature. Since hydrocortisone absorption appears to be proportional to the thickness of the stratum corneum and since the stratum corneum serves as a depot for hydrocortisone absorption it would appear that the transepidermal route is probably the major pathway for the intracutaneous absorption of hydrocortisone.

B. DIMETHYL SULFOXIDE

CHEMICAL AND PHYSICAL PROPERTIES

Pure dimethyl sulfoxide is a colorless, odorless, organic liquid (58,78) with the following structure.



It has a higher specific gravity and is slightly more viscous than water (78). The sulfur-oxygen bond in dimethyl sulfoxide is quite polar giving it a dielectric constant of 46.7 (79). This is relatively high, as methanol has a dielectric constant of 33.7 (80). Dimethyl sulfoxide has been shown to be completely miscible in all proportions with water and most organic solvents (78). It also is a versatile solvent, dissolving many inorganic and organic compounds (81,82,83). For example, one ml will dissolve 250 mg of hydrocortisone (84). As well, it has the ability to form metallic chelates (85) and complexes with metallic salts (85,86). Dimethyl sulfoxide is very hygroscopic, absorbing greater than 70 per cent of its own weight of water from air (87).

THERAPEUTIC APPLICATIONS

Dimethyl sulfoxide was first synthesized by Alexander Saytzeff in 1867 (83). This compound remained relatively obscure until the 1940's when it was successfully used as a solvent for the spinning of polyacronitrile fibres (83). The initial "medicinal" application was as a preservative in plasma and whole blood preparations to prevent cell damage during freezing (88). Clinical studies on dimethyl sulfoxide commenced in the United States in October of 1963 (89) with the first reports on its pharmacology and therapeutic value being published in 1964 (90,91).

On November 11, 1965, the United States Food and

Drug Administration (F.D.A.) recalled the New Drug application of dimethyl sulfoxide discontinuing clinical research on humans (85). This resulted because of reports of adverse effects of the drug on the eyes of experimental animals which received relatively large doses of dimethyl sulfoxide orally for long periods of time (92). Changes in the refractive index and lens changes were shown to be the adverse effects (93). A partial resumption of clinical testing was permitted by the F.D.A. on December 23, 1966 (89). On September 10, 1968 the F.D.A. allowed the resumption of the short term use of dimethyl sulfoxide (93). They suggested that the topical application of not more than one Gm per Kgm body weight for not more than 14 consecutive days would be reasonably safe.

Jacob, Bischel and Herschler (90) in their initial report on the potential of dimethyl sulfoxide as a medicinal agent suggested uses for it as:

- (a) penetrant carrier
- (b) local analgesic
- (c) anti-inflammatory adjunct
- (d) bacteriostatic agent
- (e) diuretic
- (f) tranquilizer

Its value as an analgesic, anti-inflammatory adjunct, bacteriostatic agent, diuretic and tranquilizer is quite controversial since dimethyl sulfoxide appears to offer little or no value over standard therapy in these areas. However, its use as a penetrant carrier to enhance the absorption of other molecules has received a great deal

of attention. Claims ranging from no effect to a 25 fold increase in skin penetration of some compounds in the presence of dimethyl sulfoxide have been reported. This enhancement in skin penetration would probably be of interest in dermatology since the skin barrier appears to cause the ratio of absorbed to applied drug to be very low for the vast majority of compounds.

Although dimethyl sulfoxide has been shown to increase the percutaneous absorption of many compounds not all appear to be affected in a similar manner. For example, certain studies have demonstrated that the penetration of thiopental (94), d-tubocurarine (94) and trypan blue (95) are not affected. Kastin, Arimura and Schally (96) reported that the absorption of vasopressin, melanocyte stimulating hormone and adrenocorticotropin were not enhanced to a significant degree. Groel (97) has observed that triamcinolone acetonide is no more effectively absorbed from dimethyl sulfoxide than from a cream base. This is contrary to the results obtained by Stoughton (98). These varying observations by Groel and Stoughton probably result because of the different methods of analysis employed by each.

It was first noted by Jacob, Bischel and Herschler (90) that 15 per cent aqueous solutions of dimethyl sulfoxide instilled into the bladder of a dog greatly enhanced the penetration of sodium salicylate, sodium sulfadiazine, Evans blue dye and sodium heparin, as judged by

blood levels. Since this initial observation, a wide variety of compounds have been shown to penetrate biological membranes (especially skin) to a greater degree in the presence of dimethyl sulfoxide.

Inorganic compounds such as mercuric chloride (99), picrate ion (100) and the antiperspirant agent aluminum chloride (81) have all resulted in better absorption in the presence of dimethyl sulfoxide. Kligman (81) reported that methylene blue and tetrachlorosalicylanilide, a brilliant fluorescent dye, penetrated the skin more freely when applied with dimethyl sulfoxide. A variety of chemotherapeutic agents such as declomycin (81), griseofulvin (101), penicillin G potassium (102) and 6-mercaptopurine (103) were also absorbed to a greater extent. Stoughton and Fritsch (104) in studies on the influence of dimethyl sulfoxide on human percutaneous absorption showed the absorption of hexopyrronium bromide, a quaternary compound, and naphazoline hydrochloride to be increased 25 times. Soman (105,106), an organophosphorous anticholinesterase, and salicylic acid (107), a keratolytic agent, were also observed to have increased skin penetration ability when administered with dimethyl sulfoxide.

Increased penetration also has been demonstrated for a number of steroids. Feldmann and Maibach (66,67) in two separate studies have shown percutaneous penetration of testosterone to be increased from 3.5 to 4 times with dimethyl sulfoxide. Such an increase in the absorp-

tion of testosterone has also been reported by Kligman (81). Estradiol (108) and its benzoate salt (109) have been transported through the skin barrier in increased quantities when applied with dimethyl sulfoxide. Flucinolone acetonide (98,104), triamcinolone acetonide (98) and cortisone acetate (108) have all shown increased absorption in the presence of dimethyl sulfoxide. Probably the greatest potential for dimethyl sulfoxide as a penetrant carrier lies in this area with its apparent ability to enhance the penetration of glucocorticoids through the skin barrier. This would be of significant importance as dimethyl sulfoxide may enhance the intracutaneous absorption of glucocorticoids thereby making them more available to exert their anti-inflammatory action in dermal tissue. This enhanced penetration of glucocorticoids in the presence of dimethyl sulfoxide also may be of value monetarily. Since the ratio of absorbed to applied glucocorticoid appears to be extremely low, this enhanced penetration could lead to a reduction in the dose of glucocorticoid applied topically and hence reduce the cost of these steroid preparations.

It has been pointed out by Sulzberger et al. (110) that substances incorporated in dimethyl sulfoxide can very rapidly penetrate into and be deposited in the horny layer of the skin and thus form a depot in this area. Stoughton (111) has demonstrated that dimethyl sulfoxide enhanced the deposition of ^{14}C -labeled hexachlorophene in

the horny layer from 5 to 25 times, thus increasing the bacteriostatic effectiveness in this layer of the skin. He observed that the reservoir remains at a measurable level for four or five days and that this induced reservoir of hexachlorophene can be almost completely removed by stripping off the stratum corneum. Dimethyl sulfoxide has also been shown to increase the retention of some steroids in the stratum corneum resulting in the formation of a reservoir. According to Smith and Allison (109) a portion of the estradiol-17- β -benzoate administered in dimethyl sulfoxide may have been retained in a reservoir in the stratum corneum. Stoughton (98) suggests that within five minutes after the topical application of glucocorticoids in dimethyl sulfoxide a reservoir in the stratum corneum is saturated with these glucocorticoids. One minute of exposure to 0.1 per cent triamcinolone acetate in 90 per cent dimethyl sulfoxide was found sufficient to prevent the removal of the steroid from the skin by washing with soap and water (81). The above observations suggest that the stratum corneum may preferentially retain certain compounds thereby decreasing their rate of absorption and prolonging the period over which they are absorbed. This retention of compounds in the stratum corneum supports the belief that this area of the skin serves as the barrier to transepidermal absorption. Therefore, if dimethyl sulfoxide can break down this barrier dermal absorption should be expected to increase.

MECHANISMS OF DIMETHYL SULFOXIDE IN ENHANCING ABSORPTION

The exact mechanism by which dimethyl sulfoxide enhances penetration of the skin by drug molecules is not apparently known, although many theories have been put forth. The only principles regarding its mechanism on which there appears to be fairly consistent agreement are that relatively high concentrations of dimethyl sulfoxide are required to increase absorption to any degree (100, 110,112) and that the effect of dimethyl sulfoxide on the skin is reversible (90,112,113). The ability of dimethyl sulfoxide to produce a temporary effect on the skin barrier is significant since permanent alterations in the barrier properties of the stratum corneum due to dimethyl sulfoxide would not be desirable.

Baker (113) has suggested that the speed with which barrier suppression is produced implies a direct effect of the solvent on the stratum corneum. Although it has been generally felt that dimethyl sulfoxide does exert its effect by acting on the stratum corneum to alter its barrier properties, Sulzberger et al. (110) have demonstrated that absorption may occur via the hair follicles. When working with hairless mice, Sweeney, Downes and Matoltsy (114) noted that the rate of passage of water through the skin was increased 90 times by the presence of 90 per cent dimethyl sulfoxide. These observations indicate that hair follicles may be a secondary pathway for dimethyl sulfoxide but that these follicles do not appear to be the major

route in the enhancement of absorption by dimethyl sulfoxide. Exactly how it enhances absorption is quite controversial.

Many investigators (110,113,115) postulated that since dimethyl sulfoxide is highly hygroscopic it increases the hydration of the stratum corneum. They suggested that this hydration decreases the capacity of the stratum corneum to act as a barrier and thus increases absorption. Block (83) proposed that dimethyl sulfoxide does so by picking up moisture from surrounding tissue thereby increasing the moisture content of the micro-environment of the dimethyl sulfoxide molecule which allows for a more rapid absorption through the skin.

Some investigators (107,115,116,117) have suggested that dimethyl sulfoxide dehydrates rather than hydrates the stratum corneum due to its hygroscopicity. It has been reported by Baker (115) that this would alter the physical-chemical state therefore modifying the barrier and allowing molecules to pass through. Stelzer, Calaizze and Wurdock (107) proposed that such dehydration would increase the penetration of lipid soluble drugs and would have the reverse effect on water soluble drugs. This view is somewhat contradicted in that the penetration of some water soluble drugs has been shown to be enhanced by the presence of dimethyl sulfoxide (81,99,100).

The ability of dimethyl sulfoxide to extract fat from the skin as well as hydrate it has been indicated

(110,116). Due to its exceptional solvent powers, dimethyl sulfoxide may solubilize the lipoidal material of the cell walls and thereby increase its passage as well as the passage of dissolved drug through the skin barrier (107).

It has also been proposed that increased cutaneous permeability induced by dimethyl sulfoxide resulted from modification, and perhaps, dissolution of the keratin content of cells (118).

Levine and Pelikan (119) indicated that ethylenediaminetetraacetic acid (EDTA) causes increased absorption through biological membranes by the removal of calcium from these membranes. They pointed out that the presence of calcium increases the proximity of adjacent molecules thereby decreasing permeability through solidification of the membrane. Therefore, the fact that dimethyl sulfoxide is able to form metallic chelates may be a factor to consider in the explanation of demonstrated increases in absorption rates of various compounds (83).

The "lipoperoxide permeability of membranes" hypothesis has been proposed by Muset and Martin-Estève (120). This hypothesis states, that when an unsaturated fatty acid is autoxidated or peroxidated a shift in double bonds takes place together with a modification of geometric isomerism. They postulated that since dimethyl sulfoxide has the capacity to modify the stereoisomerism of fatty acids it may, by this modification, increase the permeabil-

ity of the lipoidal layer of the skin.

Sulzberger et al. (110) noted that there was an abrupt fall off in dimethyl sulfoxide's capacity to enhance penetration in concentrations below 70 per cent. In these high concentrations of dimethyl sulfoxide microscopic analysis by Montes et al. (121) has shown tissue damage. They demonstrated that the horny layer underwent separation from the underlying granular cells, that most of the cells lost their contents and that the spaces between the cells which were normally empty became enlarged and distended. This enlargement and distension of the intercellular spaces is possibly edema which is probably due to the hygroscopic nature of dimethyl sulfoxide. Edema was noted by Skog and Wahlberg (122) as the dominant change in the skin after the application of 80 per cent dimethyl sulfoxide. They suggested that the edema was a result of a manifest effect of dimethyl sulfoxide on the blood vessels either indirectly by the liberation of histamine or by a direct effect of dimethyl sulfoxide on the vascular walls.

Wright and Winer (123) observed an increase in thickness of the stratum granulosum and stratum malpighii. This increase in thickness may be due to edema or to swelling of the protein fibres of the skin which could be the result of edema. It has been indicated by Elfbaum and Laden (124) that dimethyl sulfoxide may cause a swelling or expansion of the protein fibres in the skin which they

have suggested may result in enhanced percutaneous penetration. It was also noted by Elfbaum and Laden (100,124) that there was no marked increase in fibre swelling and absorption until relatively high concentrations of dimethyl sulfoxide were used. Rammler and Zaffaroni (112) agree that dimethyl sulfoxide exerts its effect by its action on skin protein. It has been postulated by them that the permeability of dimethyl sulfoxide through the protein barrier of the skin, whose conformational integrity is dependent upon bound water, is the result of reversible configurational changes of these proteins because of water substitution by dimethyl sulfoxide. They suggested the following: that major configurational changes occurred with concentrations of dimethyl sulfoxide between 60 and 70 per cent; that the configurational alteration is apparently reversible after removal of dimethyl sulfoxide and that the effectiveness of dimethyl sulfoxide in regard to changing the configuration of proteins appears to be related to its size and capacity to substitute for or bind with water in addition to affecting other hydrogen bonded structures. It has also been pointed out by Rammler and Zaffaroni (112) that the dimethyl sulfoxide hydrate contains 2 M of water and is formed exothermically. They indicated this suggests that the most effective concentration of this substance for topical application, barring water evaporation after application, is greater than 67 per cent. With increasing concentrations of dimethyl sulfoxide

they feel that the heat of hydration would assist in increasing the rate of diffusion across the epidermis and that the most effective concentration for topically applied dimethyl sulfoxide is 90 per cent.

The above observations suggest that dimethyl sulfoxide acts primarily by altering or damaging the integrity of the skin barrier. This is disputed by Kligman (81) who suggests that dimethyl sulfoxide does not affect permeability by violating the structural integrity of the horny layer. According to him, since the horny layer must be nearly saturated with dimethyl sulfoxide in order to achieve significant penetration, the drug is conducted through this barrier by remaining dissolved in a continuous channel of dimethyl sulfoxide which occupies the internal spaces of the horny layer. A similar mechanism has also been postulated by Misch and Misch (125).

According to Jacob and Wood (92), Franz and Van Bruggen (126) and Jacob, Wood and Brown (89) dimethyl sulfoxide by its own passage through membranes influences the movement of other compounds into the skin. This mechanism is similar to the "carrier mechanism" proposed by Morain, Replogle and Curran (127) and Jacob, Bischel and Herschler (91) whereby dimethyl sulfoxide would carry other molecules through the skin barrier. Jacob, Bischel and Herschler (91) have listed some pertinent facts believed to have a bearing on the "carrier mechanism" which include the following: dimethyl sulfoxide displays exceptional ionizing

powers when solutions of organic and inorganic compounds are considered; many organic compounds form complexes with dimethyl sulfoxide which would tend to show increased polarity and metallic salts form complexes with dimethyl sulfoxide, many of which are hygroscopic. Block (83) suggests that in the first two instances it is difficult to see how an increase in polarization would result in increased penetration through a biological membrane. As he pointed out, it is generally accepted that the less polar the molecule the more easily it passes across such a membrane. According to Block (83), passage across a biological membrane may be hastened by the formation of a transition-complex which readily dissociates into the original molecule and the carrier but, complex formation prior to reaching the membrane surface would tend to preclude transfer across the membrane. He further suggests that complex formation may allow the drug to reach the site of absorption more readily. If the salt complexes formed are hygroscopic one must also consider whether or not the shell of hydration about the complex decreases the permeability and absorption due to an increase in apparent volume of the complex (83). Elfbaum and Laden (124,128) indicated that dimethyl sulfoxide does not act by this "carrier mechanism". They demonstrated that picrate ion and dimethyl sulfoxide were transferred independently of each other therefore suggesting that dimethyl sulfoxide does not act by a direct carrier effect

or by active transfer.

With all of the controversy as to the mechanism of dimethyl sulfoxide in enhancing absorption, it appears generally that dimethyl sulfoxide influences the stratum corneum barrier. Its ability to reduce this barrier probably has more support than does the carrier mechanism. Regardless what the mechanism of dimethyl sulfoxide may be, the majority of reports indicate that large concentrations of dimethyl sulfoxide are required to influence percutaneous absorption.

C. EFFECT OF DIMETHYL SULFOXIDE ON TOPICAL HYDROCORTISONE ABSORPTION

Since hydrocortisone appears to exert its primary anti-inflammatory effect in dermal tissue, the importance of an increase in its absorption into this area would seem to be considerable. Therefore, if dimethyl sulfoxide can enhance the intracutaneous absorption of hydrocortisone, the value of dimethyl sulfoxide in the topical therapy of inflammation may be of significance.

Berliner and Ruhmann (36) in their work have noted a certain degree of synergism between dimethyl sulfoxide and corticosteroids. They indicate that dimethyl sulfoxide could be an important tool for potentiating the biological effectiveness of steroids at the cellular level. They reported that dimethyl sulfoxide reversibly depressed the growth of fibroblasts. As has been mentioned earlier, hydrocortisone is reported to be the most potent fibroblast

inhibitor among the naturally occurring corticosteroids (37). It has been shown by Gries et al. (129) that the treatment of rabbit skin with dimethyl sulfoxide results in a decrease in collagen breakdown, a decrease in the synthesis of collagen and a decrease in the synthesis of mucopolysaccharides. They also noted that antiphlogistics caused a decrease in the synthesis of collagen and mucopolysaccharides.

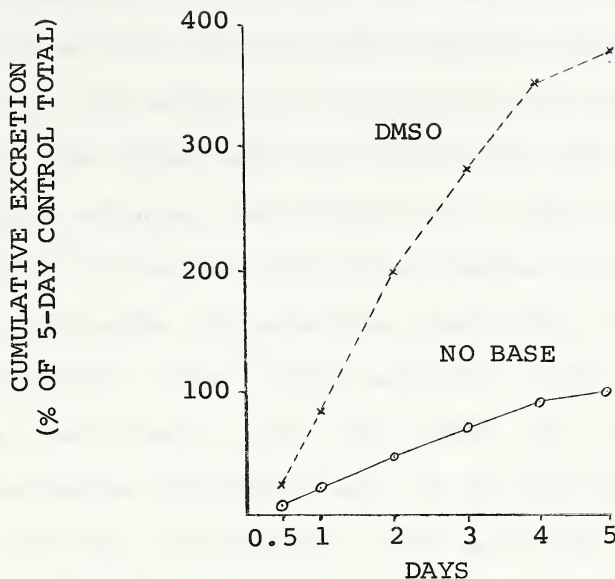
Weissmann, Sessa and Bevans (130) have suggested that dimethyl sulfoxide makes hydrocortisone more available to lysosomes in vivo thereby lowering the effective concentration of hydrocortisone necessary to stabilize the lysosomal membrane. The ability of hydrocortisone to stabilize lysosomal membranes and, by doing so retard the release of hydrolases from these lysosomes thus preventing these proteolytic enzymes from destroying cells, has been discussed previously (see p. 9).

These observations suggest that dimethyl sulfoxide may have a synergistic effect on the action of hydrocortisone as well as increasing the penetration of hydrocortisone into the skin. This two fold effect would therefore enhance the value of dimethyl sulfoxide as a vehicle in the topical application of hydrocortisone.

Although this dual effect must be considered in identifying the value of dimethyl sulfoxide in hydrocortisone topical therapy, the major effect of interest in the present work was to evaluate the penetrant effect.

This approach was of primary interest since it was desired to determine the penetrant effect of dimethyl sulfoxide on hydrocortisone from a formulation point of view.

The ability of dimethyl sulfoxide to enhance the absorption of hydrocortisone when applied topically has been well established. Work by Maibach and Feldmann (66,67,68,131) indicated that a 3.5 to a 5 fold increase in the absorption of hydrocortisone resulted when the hydrocortisone was applied with 100 per cent dimethyl sulfoxide. They (68) also observed that peak absorption of hydrocortisone occurred in the first two days. This was followed by a plateau of excretion to the sixth day which then dropped to zero by the tenth day. Dimethyl sulfoxide, as well as increasing the total excretion of hydrocortisone five times, also was shown to increase the rates of hydrocortisone absorption disproportionately in the first two days (68). It was pointed out by these same workers that the change in the configuration of the excretion curve suggested that the barrier properties of the skin had been altered. The excretion pattern has been presented by Maibach and Feldmann (66,67) in two separate studies, one of which is presented in Figure 1.



PERCUTANEOUS PENETRATION OF HYDROCORTISONE. THIS IS A CUMULATIVE EXCRETION CURVE. THE FIVE-DAY CONTROL UNTREATED AREA IS REPRESENTED AS 100%.

FIGURE 1. From FELDMANN, R.J., and MAIBACH, H.I., ARCH. DERMATOL., 94, 649 (1966).

The above observations suggest that dimethyl sulfoxide is probably of significant value in increasing topical hydrocortisone therapy.

Since the desired site of action of topically applied hydrocortisone appears to be within the skin, it also becomes of interest for dimethyl sulfoxide to increase the intracutaneous absorption of hydrocortisone without a subsequent high increase in its systemic absorption. If dimethyl sulfoxide caused a proportional increase in sys-

temic absorption, its value as a penetrant carrier would possibly be over-ruled by the probable increase in undesirable side effects of hydrocortisone absorbed systemically. The above work has shown only that dimethyl sulfoxide enhances the absorption of hydrocortisone systemically but has not indicated whether or not the dimethyl sulfoxide increased the absorption of hydrocortisone into the skin. Also, Plein and Plein (132) and Hlynka, Anderson and Riedel (133) have shown that systemic and intracutaneous absorption are not proportional.

The work of Stoughton (134) and Munro and Stoughton (101) indicates that dimethyl sulfoxide resulted in an increase in the retention of hydrocortisone in the stratum corneum. Stoughton (134) observed the rapid penetration and retention by the horny layer of hydrocortisone when administered in dimethyl sulfoxide. He noted that 15 to 100 times as much glucocorticosteroid is retained in the horny layer and that this protected reservoir is established within a few minutes after application. The reservoir was shown to remain for about 16 days and was able to be removed by scotch tape stripping. These observations by Stoughton support the stratum corneum as being the barrier to hydrocortisone absorption. Therefore, if dimethyl sulfoxide can break down this barrier to transepidermal absorption, the dermal absorption of hydrocortisone would be expected to increase.

D. METHODS OF MEASURING HYDROCORTISONE ABSORPTION

The objective of measuring absorption should be to obtain a quantitative evaluation of the rate and extent of penetration by a topically applied drug into and through the skin. A variety of methods for measuring absorption has been used with varying degrees of success. Some of these methods represent the relative drug release from the vehicle to the skin, others measure drug absorption by skin tissue (intracutaneous) but the majority measure absorption through the skin (systemic). These methods are reviewed in the following discussion according to the absorption phase which they measure.

IN VITRO METHODS

All procedures using inanimate media for measuring absorption may be classified as being in vitro methods. The determination of drug release from a vehicle either into a medium such as agar gel or through a medium such as cellulose film appears to be the most popular approach to such measurements. Models developed by Wood, Rising and Hall (135) and by Billups and Sager (136) appear to be satisfactory for measuring the release of a drug from a vehicle and consequently the effect of formulation factors which might influence this release. They are inadequate, however, for evaluating the effect which such factors may have on the subsequent absorption of the released drug by the skin. The complex chemical, physical

and biological properties of living skin precludes adequate simulation by in vitro models (137). Therefore, it is felt that percutaneous systemic and intracutaneous absorption studies must be performed on an in vivo basis.

SYSTEMIC MEASUREMENTS

A quantitative method for measuring systemic absorption should be capable of determining the rate at which a drug penetrates into the circulatory system of the body after topical application. Methods which measure penetration into the circulatory system are commonly referred to as methods for measuring percutaneous absorption. Although it may be implied that systemic absorption measurements are representative of intracutaneous absorption, it has been indicated that they do not necessarily represent intracutaneous absorption (133). In some cases, percutaneous measurements even have significant limitations as quantitative methods for measuring systemic absorption.

The appearance of pharmacological and physiological effects which are characteristic of a drug is one method which has been used to interpret percutaneous absorption of the agent. Several responses have been utilized to determine the degree of hydrocortisone absorption such as vasoconstriction (138,139), involution of the thymus gland (140), alterations in melanocyte stimulating hormone secretion (141) and decreases in the number of circulating eosinophils (6,142,143). There are several limitations to

these approaches as quantitative measurements of systemic absorption. One such limitation is that even if the drug is capable of affecting a significant response, the magnitude of such a response is difficult to calibrate and quantitative results are usually replaced by a yes-or-no answer to the question of absorption (137). Another pitfall to this approach may be encountered if the drug should be absorbed but only in a concentration below the minimum threshold required to register a response systemically (144). Finally, unless the amount of drug which is metabolized, stored and excreted is known relative to the total amount which penetrates, a quantitative evaluation of the rate of systemic absorption by this method would not be reliable (145).

Perhaps the most popular procedures used to measure systemic absorption have been the analyses of blood (6,143, 146) and urine (8,63,147) for the presence of the penetrating molecule and/or its metabolites. For example, in an attempt to determine the degree of hydrocortisone absorption, hydrocortisone itself (141) and some of its metabolites such as 17-hydroxycorticosteroids (6,147,148), 17,21-dihydroxy and 20-ketosteroids (146) and 17-ketosteroids (61,63,147,148) have been analyzed in blood and urine. Several investigators also have measured the total amount of radioactivity in the urine after the topical application of labeled hydrocortisone (8,66,67,68). Problems in measuring hydrocortisone and its metabolites are considerable

since it appears that very little hydrocortisone is absorbed percutaneously and, secondly, the hydrocortisone which is absorbed is excreted as a wide variety of metabolites. For example, less than one per cent of the hydrocortisone is excreted intact, about 40 per cent is excreted as 17-hydroxycorticosteroids and another six per cent as 17-ketosteroids (149). Therefore, in several instances only a portion of the amount being absorbed is actually being measured which makes detection difficult.

Caution also must be exercised in accepting the drug concentration in any one area as an estimate of the total drug penetration through the skin. For example, the amount of drug in the urine at any given time to be representative of the quantity of drug which penetrated through the skin must be corrected for the amount of drug which may be stored in various tissues and blood or that amount which may be metabolized. Such a problem would probably be encountered with hydrocortisone. Several investigators (150, 151, 152) have shown that about 90 per cent of the hydrocortisone in the blood is bound to plasma proteins at physiological levels. Since it appears that only a small percentage of the applied hydrocortisone is absorbed, most of it will probably be bound to these plasma proteins. Hence, urine measurements may not be an accurate measure of the amount percutaneously absorbed. Scheuplein (50) has suggested that the majority of the drug penetrating through the epidermal barrier should be removed by the rich vascularity

of the superficial layers of the dermis. Since hydrocortisone is desired in the dermal area, systemic measurements in this respect appear questionable as being representative of intracutaneous absorption.

INTRACUTANEOUS MEASUREMENTS

Methods for measuring intracutaneous absorption should be directed at determining the amount and distribution of drug in the dermal tissues at various time intervals rather than at the rate with which the drug penetrates into this phase for the following reason. The rate of entry may not necessarily represent the amount of drug in the living epidermis and dermis since some drug probably will pass from these tissues into the circulatory system. The concentration and distribution of drug in this phase are important if it is assumed that the primary objective of initiating intracutaneous absorption is to achieve the therapeutic effect of the drug in this area. With hydrocortisone therapy this seems to be the case.

A major obstacle to quantitating drug absorption by this phase is in the separation of the drug from these tissues for analysis. This obstacle may be overcome largely by the use of radioisotope labeled drugs which can be detected in the tissues without separation (153). Radioisotopes are also advantageous in that they offer a high sensitivity of measurement (154). This factor is particularly important in intracutaneous absorption investigations

where again the amount of drug absorbed as compared to the amount applied is usually very small (137).

Despite the advantages offered by isotopes, the majority of methods which have been used to measure intracutaneous absorption with this technique still appear to have limitations in providing quantitative reproducible results. Malkinson (59) has studied the penetration of ^{14}C -labeled hydrocortisone into the skin by measuring the disappearance of radioactivity from the surface. His basic procedure involved the application of the labeled drug to the skin, counting the radioactivity on the surface with a Geiger counter and then relating the decrease in surface counts at various times to the amount of drug being absorbed. Several shortcomings prevent this technique from qualifying as a quantitative, reproducible method for measuring intracutaneous absorption. Variables in counting the radioactivity on the surface may produce large experimental errors in this procedure (137). For example, the geometry or position of the subject relative to the counter must be kept constant. Also, since the amount of drug which penetrates into the skin is usually much less than that present on the surface, errors of this nature may prevent the accurate measurement of absorption (137). A loss of radioactivity from the surface could also result from the drug penetrating into the stratum corneum and into the blood system, neither of which would be representative of intracutaneous absorption.

Histological examination of skin sections for the presence of absorbed drug also has been a popular approach to measuring intracutaneous absorption (155). Although this method may be effective for determining the presence and depth of penetrant in the skin, it does not permit a quantitative evaluation of absorption (137).

Examination of skin specimens for tracers indicating drug absorption also has been used. Dyes (156,157, 158), fluorescent compounds (156,158) and radioactive isotopes (61,159,160) are among the tracers which have been used to detect drug molecules. As Blank (137) has pointed out, however, absorption data from studies using tracers should be accepted with caution since the tracer may become detached from the drug molecule during the absorption process and consequently may not be representative of the amount of drug present. Again, the use of stable radioactive-labeled drugs has largely overcome the problem of separation. The work of Scott and Kalz (76) on autoradiography exemplified the use of this technique in investigating the absorption of hydrocortisone by the skin. In this instance, autoradiography provided information on the relative rate, depth and route of absorption as well as the relative distribution within the skin tissue. As yet, however, it has not been demonstrated to be more than a qualitative method for studying intracutaneous absorption.

The most logical method for quantitatively measur-

ing intracutaneous absorption appears to be the one developed by Hlynka, Anderson and Riedel (133). It involved the use of an apparatus to control the application of a radioactive-labeled drug preparation to specific surface areas of the skin. Skin sections are recovered at successive depths from the treated specimen by slicing the skin parallel to the surface from the subcutaneous side to the epidermal surface with a cryostat microtome. The depth and extent of drug absorption is then determined by analyzing the serial sections of skin for radioactivity. Sheinaus, Christian and Sperandio (161) and Carson and Goldhamer (162,163) had previously employed a similar procedure. Results presented by the latter two research groups indicated trends differentiating treatments and controls. The variation among replicates, however, was very large, which limited the value of their procedures as quantitative methods for measuring the effects which formulation factors may have upon drug absorption by the skin. By refining the technical variables involved and by using hairless skin which permits only transepidermal absorption, Hlynka, Anderson and Riedel (133) have proposed a model which would appear to be the most effective for studying the relative effects of dimethyl sulfoxide on the intracutaneous absorption of hydrocortisone.

STATEMENT OF PROBLEM

The major objective of this investigation is to evaluate the quantitative effect of dimethyl sulfoxide on the intracutaneous absorption of hydrocortisone. This effect will be measured as a function of rest time and of dimethyl sulfoxide concentration.

EXPERIMENTAL METHODS AND DISCUSSION

A. DRUG PREPARATIONS

Hydrocortisone-1,2- ^3H was obtained in a 9:1 benzene-ethanol solution from New England Nuclear* in a specific activity of 1 mc/ml. Autoradiography of thin layer chromatographs developed in three different solvent systems revealed only one spot for the hydrocortisone-1,2- ^3H solution, indicating radiochemical purity of the solution.

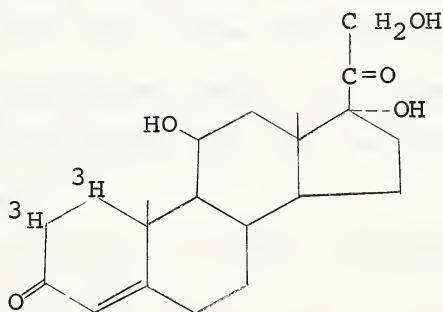


FIGURE 2: HYDROCORTISONE SHOWING POSITIONS OF ^3H LABELS

The above radioactive solution was stored in a refrigerator until it was required for incorporation into the vehicles.

A vehicle was required which would release the hydrocortisone to the surface of the skin at an adequate rate (164). Also, a vehicle containing a relatively high portion of water was desirable since this would allow the substitution of relatively high concentrations of dimethyl

*New England Nuclear Corporation, Boston, Massachusetts, U.S.A.

sulfoxide and at the same time would keep the viscosities of the various test preparations relatively constant. Aqueous Cream B.P.* appeared to meet these requirements. This oil in water cream contained 69.9 per cent water, 30 per cent Emulsifying Ointment B.P. and 0.1 per cent chlorocresol. The chlorocresol was not included in the preparations employed in this study since the preparations were being used for short periods of time, therefore, a bacteriostatic agent would not be required. Also, the chlorocresol would add another factor which may have some effect on hydrocortisone absorption. Dimethyl sulfoxide was readily incorporated into this vehicle in the concentrations used.

Five preparations containing varying concentrations of dimethyl sulfoxide ranging from 0 to 100 per cent were made. The formulae for these preparations are as follows:

PREPARATION I

Emulsifying Ointment B.P.	30%
Distilled water	69%
Dimethyl sulfoxide	0%
Hydrocortisone (stable)	1%
Hydrocortisone-1,2- ³ H solution	2 mc/Gm

PREPARATION II

Dimethyl sulfoxide	99%
Hydrocortisone	1%
Hydrocortisone-1,2- ³ H solution	1 mc/ml

*The British Pharmacopoeia, 1968, The Pharmaceutical Press, London, England.

PREPARATION III

Emulsifying Ointment B.P.	30%
Distilled water	19%
Dimethyl sulfoxide	50%
Hydrocortisone (stable)	1%
Hydrocortisone-1,2- ³ H solution	1 mc/Gm

PREPARATION IV

Emulsifying Ointment B.P.	30%
Distilled water	44%
Dimethyl sulfoxide	25%
Hydrocortisone (stable)	1%
Hydrocortisone-1,2- ³ H solution	1 mc/Gm

PREPARATION V

Emulsifying Ointment B.P.	30%
Distilled water	56.5%
Dimethyl sulfoxide	12.5%
Hydrocortisone (stable)	1%
Hydrocortisone-1,2- ³ H solution	2 mc/Gm

As can be seen in the above formulae the specific activities of Preparations I and V differ from the specific activities of Preparations II, III and IV. Preliminary studies indicated that a specific activity of 2 mc/Gm was required for Preparations I and V in order to obtain sufficient tissue counts for statistical purposes. In contrast, sufficient tissue counts were obtained from Preparations III and IV and from Preparation II when specific activities of 1 mc/Gm and 1 mc/ml were employed respectively. For purposes of discussion and data expression all of the preparations will be corrected back to a standard specific activity of 1 mc/Gm for the cream preparations and 1 mc/ml for the liquid preparation.

Preparation I was made by the following procedure. The stable hydrocortisone was dissolved in ethanol-benzene (50:50) to which the hydrocortisone-1,2-³H solution was added. This hydrocortisone solution was then added to the Emulsifying Ointment B.P. which had been previously weighed into a beaker. After removal of the ethanol and benzene by evaporation, the hydrocortisone was incorporated into the emulsifying ointment with a stirring rod. The water was then blended into the emulsifying ointment-hydrocortisone preparation. This mixture was placed between two sheets of Saran Wrap*. Manipulation of the mixture between these sheets of Saran enabled the thorough incorporation of the drug in the vehicle and at the same time reduced evaporation of the aqueous phase during the mixing procedure. The finished preparation was sealed in a five ml glass syringe and stored at room temperature.

Preparation II was prepared by dissolving the stable hydrocortisone in ethanol-benzene (50:50) in a glass vial. The hydrocortisone-1,2-³H solution was added and the ethanol and benzene were removed by evaporation. Sufficient dimethyl sulfoxide was then added to the vial to produce a one per cent hydrocortisone solution. The vial was then sealed with a rubber stopper under an aluminum cap. This preparation was again stored at room temperature.

*Saran Wrap is a registered trademark of Dow Chemical Company, Midland, Michigan, U.S.A.

The method of preparation and treatment of Preparations III, IV and V was identical to that used for Preparation I with one exception. Instead of adding 69 per cent water as in Preparation I the various concentrations of water and dimethyl sulfoxide were substituted.

The radiochemical stability of tritiated compounds becomes questionable due to the possibility of the tritium exchanging with stable hydrogen atoms in its surrounding medium. Therefore, samples of Preparation I and Preparation II were chromatographed on thin layer plates in three solvent systems. Autoradiography of these plates has shown only one spot to be present. This indicates that these preparations were radiochemically stable over the period of investigation. Both preparations were allowed to sit for at least two months before the radiochemical analysis was performed.

B. SELECTION OF ANIMALS

For this study the guinea pig was selected as the animal of choice due to the presence of hairless areas or "bald spots" of skin behind the ears of these animals. The presence of these bald areas of skin enabled the study of the effect of dimethyl sulfoxide on the transepidermal absorption of hydrocortisone since the pilosebaceous apparatus was absent.

White, male mongrel guinea pigs weighing between 650 and 800 Gm were used in all of the experiments. The

weight of each animal was determined and recorded immediately prior to use. The animals were fed commercially available pellets which were supplemented with greens or ascorbic acid. The "bald spots" are located behind each ear. Zackheim and Langs (165) have described these areas in detail. They reported that the bald spots were 1.0 to 1.5 cm in diameter and that they tended to be larger in albinos than in colored animals. Both "bald spots" on each animal were used for this study. Any "bald spot" with hairs on it was rejected. Hlynka, Anderson and Riedel (133) found the combined thickness of the stratum corneum and the remainder of the epidermis to vary from approximately 80 to 100 microns, while the dermis was about 0.6 to 1.0 mm. Upon slicing the samples parallel to the surface it was noted that major networks of blood vessels were present in the subcutaneous regions. The absence of sweat glands in the skin of the guinea pig has also been reported (166).

C. PREPARATION OF ANIMALS FOR DRUG APPLICATION

Animals were sedated at the beginning of each experiment to enable the preparation of the skin and the application of the medicament to the surface of the "bald spots". The inhalation technique used was similar to the one introduced by Hagen and Hagen (167) and later described by Hlynka, Anderson and Riedel (133). The animal was first rendered unconscious with anesthetic ether.

Anesthesia was maintained by passing a stream of air into liquid methoxyfluorane which, upon volatilizing was inhaled by the guinea pig.

After the animal was anesthetized, the hair surrounding the bald spots was removed with an Oster^{*} small animal electric clipper. A number 10 blade was used for coarse clipping. Finer clipping was done with a number 40 blade. The areas prepared for application were examined and any application site with abrasions or scars on it was excluded from the study.

A device similar to the one employed by Hlynka, Anderson and Riedel (133) was used to confine the application of the drug preparation to a specific surface area on the skin. Such a device was employed since Higuchi (46) proposed that the degree of absorption varied according to the area receiving application. Teflon cylinders (Figure 3 (B)) used to restrict the area of application were attached to the skin over the intended sites of application with household cement^{**}. A flange located near the base of the cylinder enabled contact with the skin surface. Circles two cm in diameter were marked around each "bald spot" with the aid of the open end of a test tube and an inked stamp pad. Cement was applied to the bottom of the flange and to the skin around the circum-

*Oster is the registered trademark of John Oster Manufacturing Co., Milwaukee, Wisconsin, U.S.A.

**Lepage's Pres-tite[®] Contact Cement, a product of Lepage's Ltd., Canada.

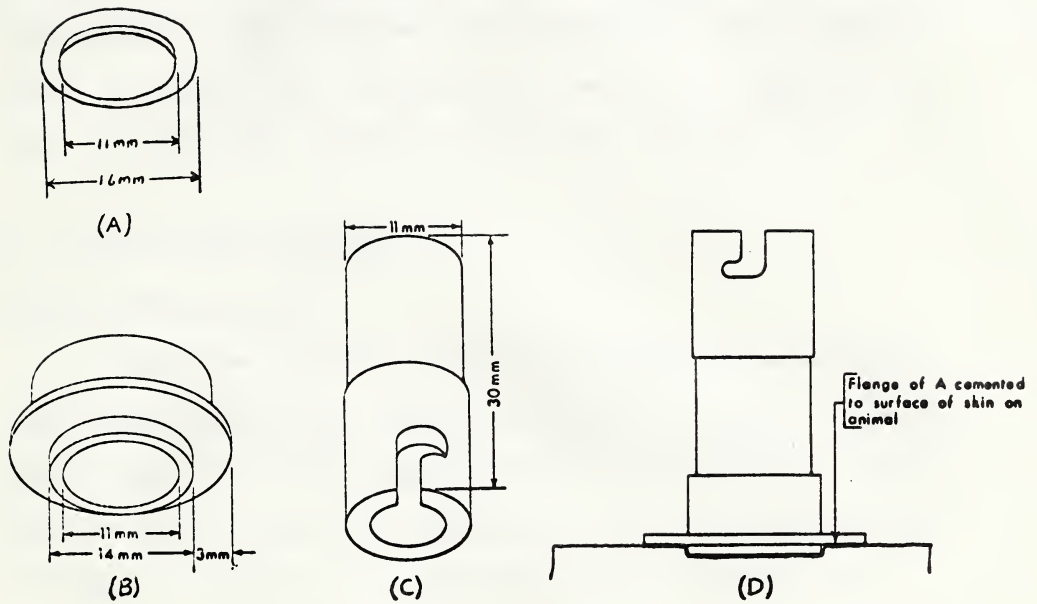


Figure 3: Detailed drawing of applicator assembly:
(A) cap; (B) cylinder; (C) applicator;
(D) assembly in position.

ference of the circle so marked. The cylinders were firmly attached to the circles marked on the skin within about one minute after cementing. A specific surface area (11 mm diameter) for application in the center of each cylinder was provided by this arrangement. The drug preparations used were introduced on teflon applicators (Figure 3 (C)) through the center of these cylinders and onto the surface of the skin (Figure 3 (D)).

D. APPLICATION OF DRUG

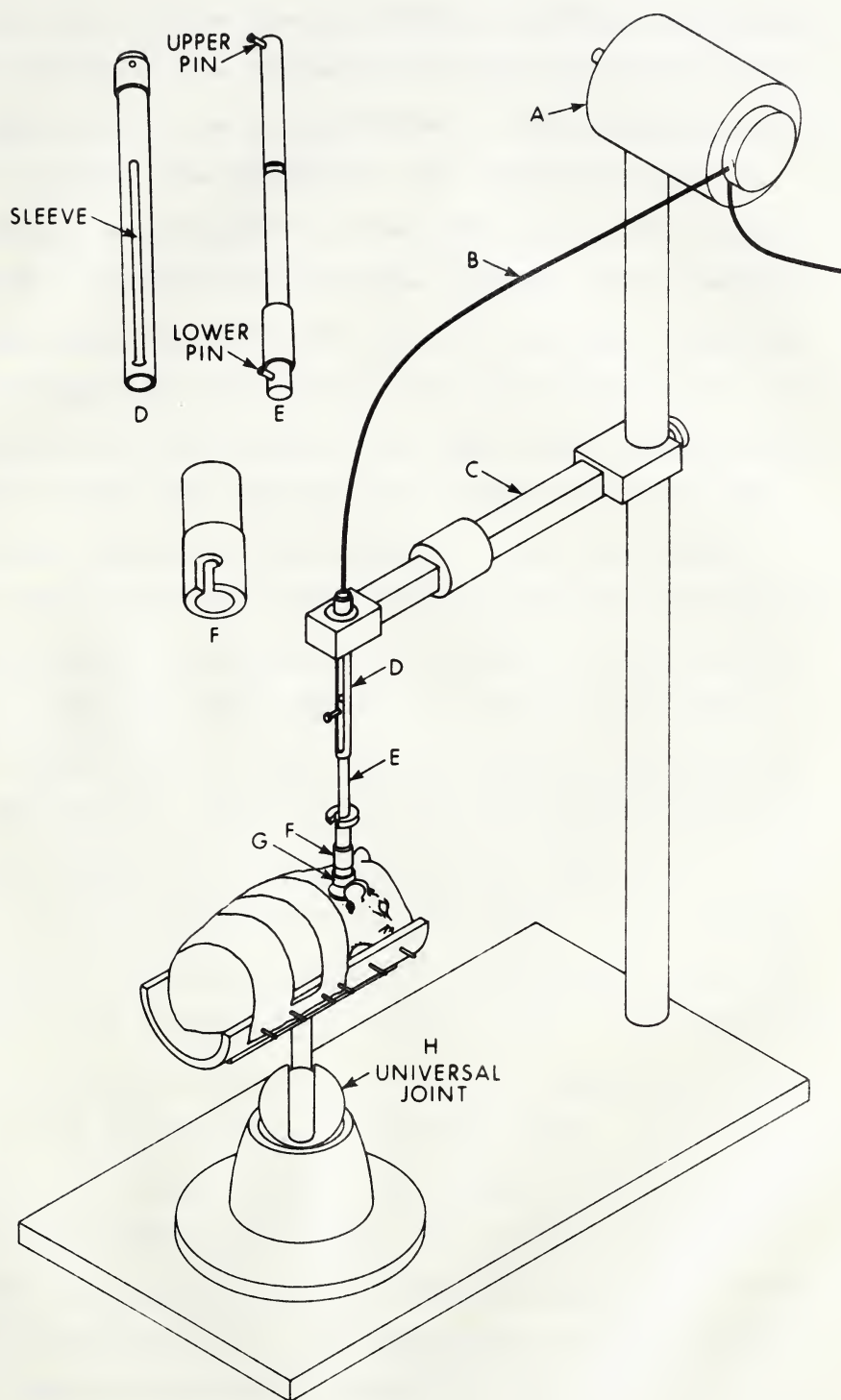
CREAM PREPARATIONS

Since the majority of vehicles used in dermatological preparations are relatively viscous, Hlynka, Anderson and Riedel (133) have suggested that some degree of rubbing be used during the application of such preparations in order to obtain a satisfactory contact between the applied drug and the surface of the skin. They also have shown that 60 second rubbing had a greater influence on systemic absorption than did 10 second rubbing while both rubbing times were found to promote intracutaneous absorption to approximately the same degree. Therefore, the latter rubbing time was used in the present study.

The apparatus used to produce uniform rubbing (Figure 4) was the same as the one developed by Hlynka, Anderson and Riedel (133) which was described as follows. A motor (A) rotated a flexible cable drive (B) which in turn rotated an aluminum rod (E). A rod inside housing

404.17

Figure 4: Drawing of complete application apparatus
with animal in position.



(D) was able to move freely through a distance determined by the length of the sleeve cut through the housing wall. The upper pin on the rod prevented it from disengaging at this point. The applicator (F) containing the drug preparation was attached to the lower end of the rod by means of a pin provided for this purpose. This assembly was then positioned directly over the cylinder cemented to the animal (G) and lowered into the cylinder until the preparation on the bottom of the applicator contacted the surface of the skin and the full weight of the rod and applicator rested on this surface. A ball and socket joint (H) on the base of the cradle permitted the accurate positioning of the cradle and the animal to achieve the proper angle for the applicator to enter the cylinder.

Preparations I, III, IV and V were all applied in the same manner. Twenty $\pm$ 0.5 mg was ejected from the storage syringe onto the surface of the applicator. This amount of drug preparation has been shown to be slightly in excess of the amount required to completely cover the exposed 11 mm diameter skin surface (133). The applicator with the drug was attached to the above apparatus and inserted into the cylinder as described above. The free rotation of the cylinders on the surface of the skin while at the same time resisting the loss of drug preparation between the applicator and cylinder was permitted by the closely machined fit of the applicators in the cylinders. Motor induced rotational rubbing was then carried out for

10 seconds at 30 r.p.m. A constant rubbing pressure was maintained with the "floating" rod assembly which absorbed slight movements by the animal in a vertical direction. After the rubbing was completed the applicator was removed from the cylinder. The cream adhering to the applicator was removed and carefully added to the inside of the cylinder. The animal was removed from the cradle and teflon caps (Figure 3 (A)) were attached to the cylinders to prevent evaporation of the dimethyl sulfoxide and water. These caps were attached firmly to the cylinders with strips of adhesive tape*. The animal was then removed from the anesthetic and allowed to regain consciousness.

LIQUID PREPARATION

Due to the relatively low viscosity of the 100 per cent dimethyl sulfoxide preparation, gauze** was used to maintain continuous contact between the applied preparation and the surface of the skin. If the gauze was not employed the preparation tended to accumulate near the edges of the cylinder. A piece of gauze 11 mm in diameter was placed inside each cylinder against the skin surface. Twenty microliters of the drug preparation was applied to the gauze using a 50 microliter syringe. The

*Elastoplast® Plastic Adhesive Strapping B.P.C., a product of T.J. Smith and Nephew Ltd., Hull and Welwyn Garden City, England.

**First-aid Sterilized Gauze Bandage, 44 x 40 mesh, a product of Rexall Drug Co., England.

caps were attached and the animal was allowed to regain consciousness.

E. REST TIME

The time in which each drug preparation remained in contact with the surface of the skin was controlled since it has been shown that absorption is a function of this contact time (168). The rest time in this study was taken as the period between the time of application and the time of sacrificing the animal. Although some additional absorption may have taken place during the time required to prepare the skin samples for analysis after sacrificing, this time was considered as being constant among replicates and was not regarded as being part of the rest period.

Preliminary studies revealed that when rest periods of less than two hours were employed large variations in results were obtained, especially from the preparations containing dimethyl sulfoxide. Since it was felt that little information would have been acquired from such measurements, rest times of less than two hours were not used in the present study.

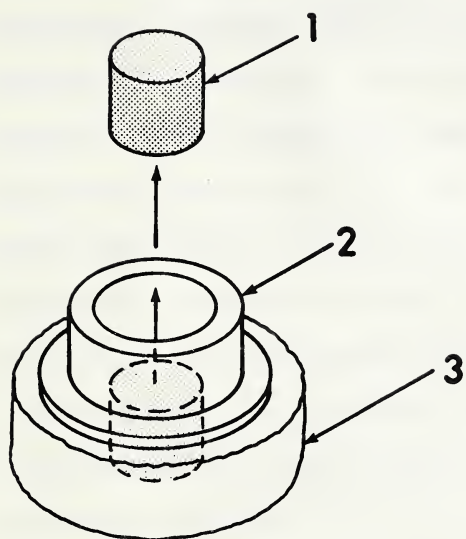
F. PREPARATION OF SKIN SAMPLES

To permit a quantitative comparison of the relative total absorption by skin samples from different experiments, skin sections, parallel to the surface of the

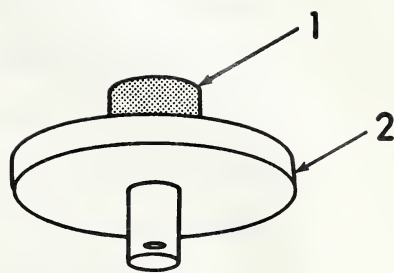
skin and located at successive depths below the surface were recovered and analyzed for radioactive hydrocortisone.

The method employed in the present study was similar to the one developed by Hlynka, Anderson and Riedel (133). The animal was re-anesthetized and sacrificed with an overdose of chloroform. The treated sites were then immediately removed from the animal by cutting the skin around the cylinders. This resulted in cylinders being attached to a circular skin sample. The caps were removed from the cylinders. This cylinder-skin combination was frozen, subcutaneous side down, for five minutes on the quick freeze block of a microtome cryostat*. The frozen assembly was then allowed to sit in the cabinet of the cryostat for another 30 minutes so it would become thoroughly frozen. This frozen assembly was then inverted, subcutaneous side up, on a lead block which was placed inside the cryostat. A punch of skin, eight mm in diameter, was removed through the center of the cylinder with a cutaneous punch (Figure 5 (A)). The skin punch obtained was placed subcutaneous side up on several drops of water on a specimen holder plate. This punch of skin was then rapidly frozen to the plate (Figure 5 (B)) on the quick freeze block. The plate holding the punch was adjusted and secured on the micro-

* International-Harris Model C T microtome cryostat, International Equipment Co., Needham Heights, Mass., U.S.A.



(A)



(B)

Figure 5: Details of: (A) Skin punch (1) recovered through cylinder (2) from skin (3); (B) Skin punch (1) on holder plate (2).

tome in such a position that the cutting blade* would slice parallel to the surface of the skin sample. The skin punch was then completely sliced into 50 micron sections until the ice below the surface was reached. These sections obtained represented decreasing depths from the epidermal surface. The proximity of each section to the epidermal surface was calculated by relating the section number to the subsequent number of 50 micron slices required to reach the ice. The two sections recovered immediately before reaching the surface were discarded as they were considered to be contaminated by the drug preparation on the epidermal surface. If skin punches became detached from the specimen holder plate before sections 100 microns from the epidermal surface were obtained, they were excluded from this study. One hundred microns of skin was placed into each of five serially number liquid scintillation counting vials** which contained one ml of digesting solution (see p. 63). The vials containing the tissue and digesting solution were then capped. The contents were digested at 70°C for three and one half hours. Twenty ml of fluor solution (see p. 64) was added and the samples were ready for counting. The 500 microns of tissue analyzed was interpreted as containing the relative amount of hydrocortisone present in the dermal area for

* Razor blades were used for slicing skin sections in all experiments. After each section, the blade was wiped off and it was changed for each sample.

** Standard 20 ml volume vials, Nuclear-Chicago Corp., Des Plaines, Illinois, U.S.A.

each replicate at each rest time.

G. PREPARATION OF BLOOD SAMPLES

Systemic absorption was measured in the initial experiments of the present study to see if the intracutaneous absorption of hydrocortisone was proportional to its systemic absorption. Since plasma proteins have a high binding affinity for cortisol (169,170) and, since no other suitable method appeared available to measure systemic absorption in the experimental animal used blood plasma analysis was employed to measure the systemic absorption of hydrocortisone.

Immediately after the rest period three to five ml of blood was removed from the animal by cardiac puncture. The blood was centrifuged at 2,000 r.p.m. for 30 minutes. This was done immediately after removal from the animal since Beisel, DiRaimondo and Forsham (170) reported that if whole blood is permitted to stand red blood cells absorb increasing amounts of hydrocortisone. Two 0.5 ml samples of plasma were then pipetted into separate scintillation counting vials. These samples were left to digest at room temperature for 12 hours after the addition of digesting agent (see p. 63) to each. Fifteen ml of fluor solution (see p. 64) was added to each sample in preparation for counting.

H. UNIFORMITY OF HYDROCORTISONE PREPARATIONS

After the cream preparations were added to both applicators in any given experiment (two applicators per animal), a five mg sample of the preparation was obtained. The sample was weighed into a scintillation counting vial to which 20 ml of fluor solution was added volumetrically. Three, one ml aliquots of the above solution were transferred with a volumetric pipette to individual scintillation counting vials. Nineteen ml of fluor solution (see p. 64) was added to each of these three vials before counting. This latter dilution was necessary since the specific activity of each preparation was very high and could not be recorded satisfactorily by the liquid scintillation counter* and corresponding computer program.

One, five microliter sample was obtained from Preparation II on every day that this preparation was employed. More frequent sampling of this preparation was not deemed necessary since it was in the form of a liquid and therefore there would be less possibility of changes in uniformity. Each sample was treated in the same manner as were the samples from the cream preparations.

The average specific activities together with the standard deviations of the various preparations were as follows:

Preparation I (22 samples) 2.452 ± 0.179 mc/Gm

*Picker Nuclear Liquimat 200, a product of Picker Nuclear, White Plains, New York.

Preparation II (6 samples)	1.130 ± 0.074 mc/ml
Preparation III (24 samples)	0.947 ± 0.049 mc/Gm
Preparation IV (24 samples)	1.093 ± 0.064 mc/Gm
Preparation V (19 samples)	2.150 ± 0.292 mc/Gm

I. RELEASE OF HYDROCORTISONE FROM THE DRUG PREPARATIONS

The purpose of measuring in vitro release was to determine the effect of various concentrations of dimethyl sulfoxide on the release of hydrocortisone from the cream preparations used in the present study. To study this effect plexiglass* cells were employed. These cells were similar to the ones used by Patel and Foss (171). Each cell consists of two plexiglass blocks and is 3 x 3 x 1 1/2 in. with four 3/16 in. holes at the corners. The cell has a cavity with about a 20 ml capacity. The method used was similar to the one described by Wood, Rising and Hall (135).

One per cent hydrocortisone creams with specific activities of approximately 3 uc/Gm containing 0, 12.5, 25 and 50 per cent dimethyl sulfoxide were prepared by the method previously described (see p. 46). The specific activity of each cream was determined by weighing three 20 mg samples into scintillation counting vials, dissolving the samples in 20 ml of fluor solution and counting.

One compartment of the plexiglass cell was filled

* Methyl methacrylate. Marketed as plexiglass by Rohm and Haas Co., Philadelphia, Pa., U.S.A.

with cream while Emulsifying Ointment B.P. was placed in the other compartment. A double cellulose* membrane, previously moistened with distilled water and blotted between two sheets of absorbent paper, was then placed between the cream and ointment surfaces. The two plexiglass blocks were held firmly together with four stainless steel head bolts, 3 1/2 x 3/16 in., inserted in the corner holes and secured with stainless steel wingnuts. These cells were allowed to stand at room temperature during which time diffusion was expected to occur from the hydrocortisone cream through the double cellulose membrane into the compartment containing the emulsifying ointment. In this study emulsifying ointment, which constitutes 30 per cent of each of the above preparations, was added to the second compartment instead of using the same cream base in both compartments as Wood, Rising and Hall (135) have done. This variation was used so that the effect of dimethyl sulfoxide and water in the cream preparations on hydrocortisone release could be determined. The double cellulose membrane was required to maintain a stable boundary condition and also to permit separation of the two parts of the semi-solid diffusion system which might cause cross contamination.

At the end of the desired diffusion times the compartments and double membrane were separated. The

*Dializer tubing, seamless cellulose, catalogue number 8-667-2, supplied by Fisher Scientific Co. Ltd., Pittsburgh, Pennsylvania, U.S.A.

ointment on the membrane facing the emulsifying ointment was removed and added to a glass container. The emulsifying ointment was also removed from its compartment and this compartment was washed with dimethyl sulfoxide. The emulsifying ointment and washings were both added to the above glass container. About 20 ml of dimethyl sulfoxide was added to this container and the contents were thoroughly mixed which resulted in a fairly fluid preparation. Three, one Gm samples were weighed into scintillation counting vials and dissolved in 20 ml of fluor solution. These samples were then ready for counting.

Each of the four preparations was analyzed in triplicate at two and sixteen hour time periods.

J. REAGENTS USED

The skin sections were digested in a 2 N solution of potassium hydroxide in methanol. This solution was stored at room temperature. The U.S.P. XVII method for standardizing 0.5 N alcoholic potassium hydroxide was used approximately once a month to check the normality of the above solution.

Five drops of 30 per cent hydrogen peroxide were employed to bleach the plasma samples. These samples were then digested with four ml of NCS*, a quaternary ammonium hydroxide reagent.

*NCS is a registered trademark of the Nuclear-Chicago Corp., Des Plaines, Illinois, U.S.A.

The fluor solution used by Hlynka, Anderson and Riedel (133) was employed for the skin, drug preparation and diffusion study samples. It was stored in a refrigerator. A primary scintillation fluor, 2,5-diphenyl-oxazole (PPO), and a secondary scintillation fluor, p-bis {2-(5-phenyloxazolyl)}-benzene (POPOP), were employed in the solution. In order to increase the miscibility of the skin tissue with the fluor solution a secondary solvent, ethylene glycol monobutyl ether (butyl cellosolve), was added to the primary solvent toluene. The formula for this fluor solution is as follows:

Toluene	- 850 ml
Butyl cellosolve	- 150 ml
PPO	- 4.00 Gm
POPOP	- 0.05 Gm

For the plasma digest the butyl cellosolve was replaced by an equal volume of toluene. This was done since the plasma digest was miscible with the fluor solution when butyl cellosolve was not present.

K. RADIOASSAY OF ³H-LABELED HYDROCORTISONE

A Picker Nuclear liquimat 220 liquid scintillation counter was used to count the radioactivity in all of the samples. The radioactivity gave a measure of the concentration of drug in each sample. Varying degrees of quenching were produced by the skin tissue, plasma, drug vehicle, digesting agents and diffusion study samples. Therefore, the external standard channels ratio technique was utilized to correct all counts obtained to corrected

counts per minute. All calculations were performed by a Digital PDP 8/L* computer. Ten minute background counts were made on drug preparation, skin tissue, plasma and diffusion study samples. The background samples used were prepared in an identical manner to the samples in the experiments on each respective medium with the exception that the radioactive hydrocortisone was omitted. Each time a series of samples was counted the background samples were also counted. The background counts were corrected for quenching and subtracted from the respective sample counts to give the corrected counts per minute of the sample. All samples were counted for ten minutes or to a pre-set count of 1,000,000, whichever was reached first.

Additional correction factors were applied to the corrected counts per minute of the tissue and plasma samples. Variations in the specific activities existed within each preparation and between the different preparations. Therefore, all tissue and plasma samples were corrected to a constant specific activity which was based on each preparation having a specific activity of 1 mc/Gm or 1 mc/ml. The specific activity used to correct the tissue and plasma samples when the cream preparations were applied was taken as the average activities of two drug preparation samples obtained during application.

*Digital Equipment Corporation, Maynard, Massachusetts, U.S.A.

This average specific activity was used for the correction of the skin and plasma samples. When the liquid preparation was employed, the correction factor was the average of the six drug preparation samples analyzed. This factor was used since Preparation II, being a solution, was expected to be homogeneous.

One other correction factor was applied to the plasma samples. The specific activity in only 0.5 ml of plasma was represented by the corrected counts per minute of these samples. Since animals have different plasma volumes, such specific activities would not be expected to represent the relative degree of systemic absorption among the animals. Reports have indicated that the plasma volumes of guinea pigs vary according to the weight of the animal (172,173). The combined results of the two groups gave a 3.9 ml per 100 Gm body weight value for the average plasma volume of male guinea pigs (172). Since the average weight of the animals used in the present study was 690 Gm, the average plasma volume would therefore be 26.9 ml. To correct for the effect of the systemic dilution of hydrocortisone due to varying plasma volumes, the above corrected counts per minute of each plasma sample was multiplied therefore by the calculated plasma volume of the test animal in ml/26.9.

RESULTS AND DISCUSSION

A. EFFECT OF DIMETHYL SULFOXIDE ON INTRACUTANEOUS AND SUBSEQUENT SYSTEMIC ABSORPTION OF HYDROCORTISONE

The intracutaneous as well as the systemic absorption of hydrocortisone was investigated to see if hydrocortisone could be detected in either of these two phases. It also was desirable to see if dimethyl sulfoxide influences such absorption. Previous work (60,61,67) has indicated that very little hydrocortisone was absorbed percutaneously in the first 12 hours after topical application. Therefore, it also was of interest to see if significant absorption into either phase with or without dimethyl sulfoxide occurred earlier than 12 hours.

Experiment I was performed to see if detectable amounts of hydrocortisone were absorbed intracutaneously without dimethyl sulfoxide and to determine the time element involved. Preparation I (see p. 44) which contained one per cent hydrocortisone and 70 per cent water was used. Absorption studies were performed at different rest times so that the relative amount of hydrocortisone absorbed at various time intervals could be compared. Two hours was the minimum rest time employed for reasons which have been discussed earlier (see p. 55). Four, eight and 16 hours were selected as the other rest periods as these times would develop the pattern of intracutaneous absorption over a reasonable time period for hydrocortisone to exert its anti-inflammatory effect.

The results of this experiment (Table I) show that significantly detectable amounts of hydrocortisone are absorbed within two hours after topical application. Also hydrocortisone appears to build up very slowly in the dermis since the intracutaneous levels gradually increased during two to 16 hours, reaching a maximum at 16 hours. This experiment indicates that hydrocortisone was absorbed intracutaneously in satisfactory amounts for measurement and at times much earlier than reported by previous workers who used systemic methods of analyses.

The next objective was to determine the effect of dimethyl sulfoxide on the intracutaneous absorption of hydrocortisone. Therefore, pure dimethyl sulfoxide containing one per cent hydrocortisone (Preparation II) was applied in Experiment II for the same rest periods used in Experiment I. As can be seen from the results of this experiment (Table II) there is a much higher amount of hydrocortisone in the dermis after two hours than resulted in Experiment I. This level rapidly declined at four hours and was followed by a gradual decrease in tissue levels of hydrocortisone which reached a minimum at 16 hours. These results appear to satisfy the objectives of seeing whether the intracutaneous absorption of hydrocortisone could be analyzed by this approach, whether dimethyl sulfoxide had a significant effect on such intracutaneous absorption and whether such absorption could be detected earlier than 12 hours after topical applica-

TABLE I
INTRACUTANEOUS ABSORPTION OF ^3H -LABELED
HYDROCORTISONE FROM A 0% DIMETHYL
SULFOXIDE PREPARATION¹

REPLICATES	ABSORPTION ² (CORRECTED COUNTS PER MINUTE)			
	REST TIME (HOURS)			
	2	4	8	16
1	1,869	1,682	1,148	2,208
2	3,630	2,471	1,753	3,696
3	1,007	2,175	4,689	2,360
4	2,049	1,783	1,806	2,517
5	584	2,606	1,279	2,098
6	2,199	1,868	3,795	4,017
Average	1,890	2,098	2,412	2,815
Standard Deviation	$\pm 1,062$	± 382	$\pm 1,468$	± 826

¹ One per cent concentration, 1 mc/Gm specific activity (corrected) hydrocortisone in Aqueous Cream base.

² Activity of absorbed hydrocortisone in guinea pig dermal tissue.

TABLE II
INTRACUTANEOUS ABSORPTION OF ^3H -LABELED
HYDROCORTISONE FROM A 100% DIMETHYL
SULFOXIDE PREPARATION¹

REPLICATES	ABSORPTION ² (CORRECTED COUNTS PER MINUTE)			
	REST TIME (HOURS)			
	2	4	8	16
1	145,188	43,758	26,280	10,594
2	153,157	33,267	37,124	11,665
3	122,194	42,017	32,857	17,345
4	118,970	39,551	35,594	12,866
5	111,343	47,622	37,513	10,550
6	125,777	38,647	30,653	12,993
Average	129,438	40,810	33,337	12,669
Standard Deviation	$\pm 16,208$	$\pm 4,893$	$\pm 4,338$	$\pm 2,522$

¹ One per cent concentration, 1 mc/ml specific activity (corrected) hydrocortisone in 100 per cent dimethyl sulfoxide.

² Activity of absorbed hydrocortisone in guinea pig dermal tissue.

tion.

Since systemic measurements were the primary methods found in the literature for determining similar absorption, it was decided that the intracutaneous measurements obtained should be compared with systemic values. Although the limitations of plasma measurements representing systemic absorption of hydrocortisone are recognized, plasma was analyzed at the four rest times to monitor a trend of systemic radioactivity resulting from the topical application of Preparations I and II. These results are presented in Tables III and IV respectively. No detectable hydrocortisone had been absorbed systemically from Preparation I until after four hours. There was activity in the plasma after eight hours which increased to a maximum by 16 hours. Preparation II on the other-hand resulted in a relatively high level of activity in the plasma after two hours, reaching a peak in eight hours and then declining at 16 hours.

To illustrate the relative effects of dimethyl sulfoxide on the intracutaneous absorption of hydrocortisone and on the trends in systemic absorption the results of Tables I and II and Tables III and IV have been represented graphically in Figures 6 and 7 respectively.

As is shown in Figure 6, dimethyl sulfoxide has caused a very substantial increase in the intracutaneous absorption of hydrocortisone. The effect of dimethyl

TABLE III
SYSTEMIC ABSORPTION OF ^3H -LABELED
HYDROCORTISONE FROM A 0% DIMETHYL
SULFOXIDE PREPARATION¹

REPLICATES	ABSORPTION ² (CORRECTED COUNTS PER MINUTE)			
	REST TIME (HOURS)			
	2	4	8	16
1	0	0	0	120
2	0	0	371	437
3	0	0	210	477
Average	0	0	194	345
Standard Deviation	<u>+0</u>	<u>+0</u>	<u>+186</u>	<u>+196</u>

¹ One per cent concentration, 1 mc/Gm specific activity (corrected) hydrocortisone in Aqueous Cream base.

² Activity of absorbed hydrocortisone in 0.5 ml guinea pig plasma samples.

TABLE IV
SYSTEMIC ABSORPTION OF ^3H -LABELED
HYDROCORTISONE FROM A 100% DIMETHYL
SULFOXIDE PREPARATION¹

REPLICATES	ABSORPTION ² (CORRECTED COUNTS PER MINUTE)			
	REST TIME (HOURS)			
	2	4	8	16
1	2,836	9,823	8,935	3,868
2	4,238	7,881	7,906	6,044
3	2,902	6,116	11,057	7,604
Average	3,325	7,940	9,299	5,839
Standard Deviation	± 791	$\pm 1,854$	$\pm 1,607$	$\pm 1,876$

¹ One per cent concentration, 1 mc/ml specific activity (corrected) hydrocortisone in 100 per cent dimethyl sulfoxide.

² Activity of absorbed hydrocortisone in 0.5 ml guinea pig plasma samples.

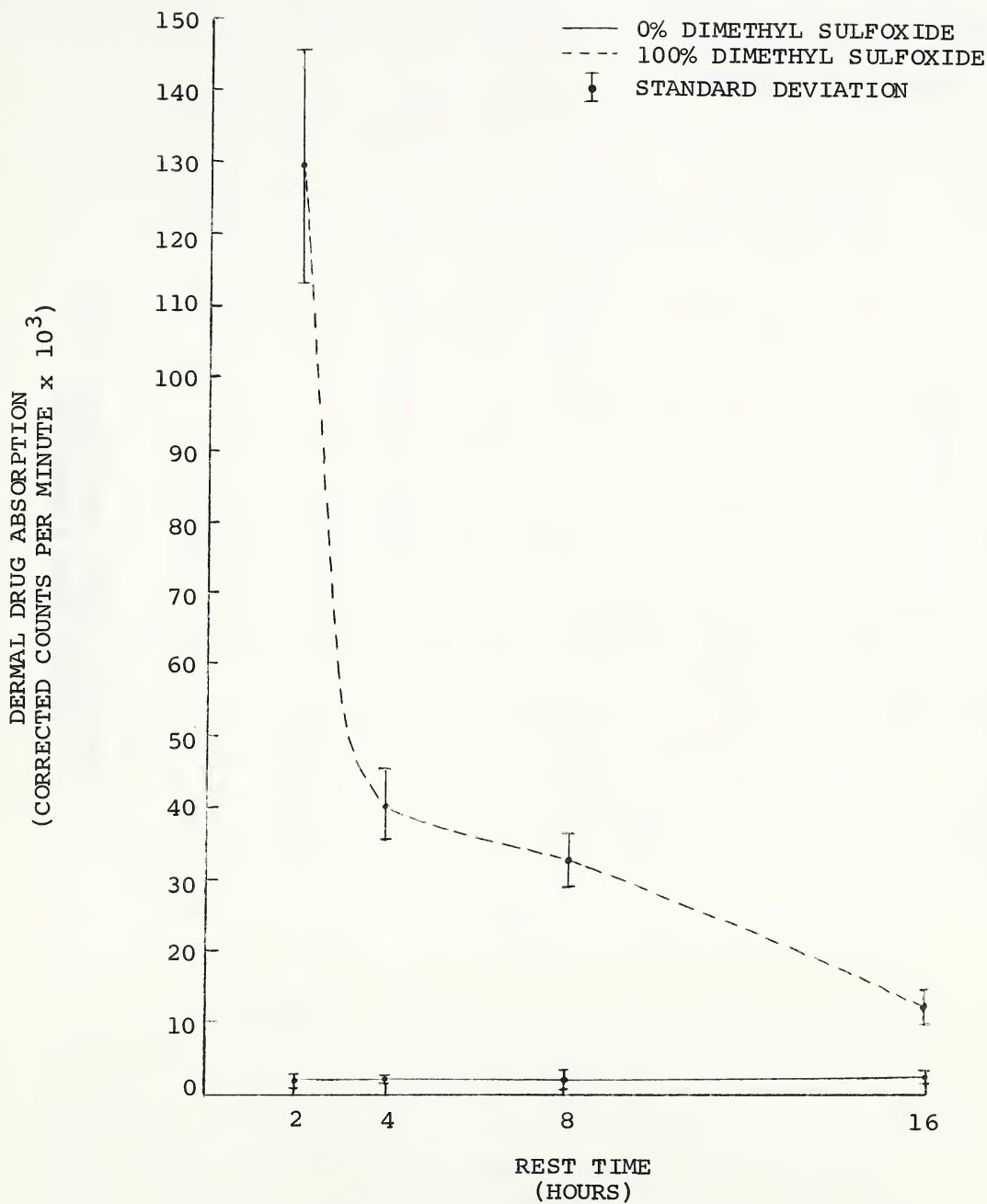


FIGURE 6: EFFECT OF 100% DIMETHYL SULFOXIDE ON THE INTRACUTANEOUS ABSORPTION OF ^3H -LABELED HYDROCORTISONE

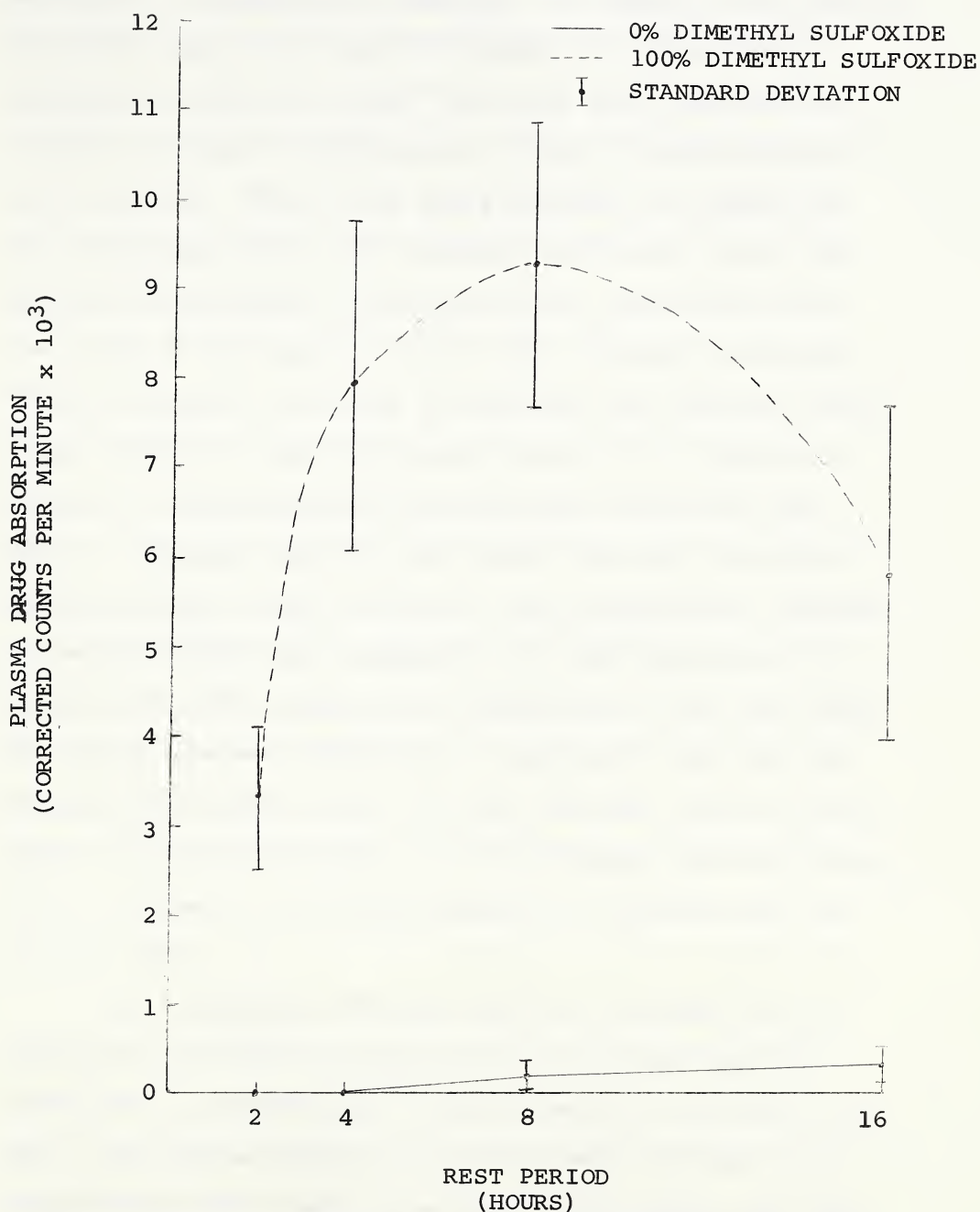


FIGURE 7: EFFECT OF 100% DIMETHYL SULFOXIDE ON THE SYSTEMIC ABSORPTION OF ^3H -LABELED HYDROCORTISONE

sulfoxide is immediately apparent. It appears that dimethyl sulfoxide exerts its major influence on intracutaneous absorption within the first two hours after application, causing an almost instantaneous influx of hydrocortisone into the skin. This is in sharp contrast to reports in the literature which show dimethyl sulfoxide causes the greatest enhancement of hydrocortisone penetration over the first 48 hour period (66,67,68). Dimethyl sulfoxide does not appear to be able to maintain the initially high dermal levels of hydrocortisone however, as a rapid decrease in intracutaneous hydrocortisone occurs by four hours. Although there is this rapid decrease in dermal hydrocortisone, there is still a very significant increase in dermal levels when compared to the zero per cent dimethyl sulfoxide preparation (Preparation I) at this time. The least apparent difference in absorption from the two preparations occurs after 16 hours although there is still about a 4.5 fold increase in intracutaneous hydrocortisone from the dimethyl sulfoxide preparation (Preparation II) at this time.

The literature indicates that the increase in the absorption of hydrocortisone with and without dimethyl sulfoxide is proportional to rest time up to two days (67, 68). This proportionality is fairly well followed with Preparation I but there is no such relationship with Preparation II. The intracutaneous results from the dimethyl sulfoxide preparation (Preparation II) indeed show intra-

cutaneous hydrocortisone to be indirectly proportional to rest time. The important feature of these results, however, is that the increase in hydrocortisone in the dermis due to Preparation II is very significant very soon after application. Therefore, the ability of dimethyl sulfoxide to promote hydrocortisone availability in this area appears promising.

As indicated by Figure 7, Preparation II also resulted in a very substantial increase in systemic absorption over Preparation I. This increase in systemic hydrocortisone absorption may be undesirable since it may enhance the systemic side effects observed after the oral administration of glucocorticoids.

By comparing the zero per cent dimethyl sulfoxide curves for intracutaneous and systemic absorption in Figures 6 and 7 respectively, it will be observed that systemic hydrocortisone was not detected until after four hours whereas significant absorption was detected in the skin tissues within two hours after application. This would indicate that the intracutaneous measurement is the more sensitive of the two methods of analysis for detecting the rate of absorption. In this respect the systemic phase would become representative of absorption through the skin only after a steady state of diffusion occurred across the dermal phase. Consequently, intracutaneous measurements appear to be the method of choice for studying topical hydrocortisone absorption, at least for the initial few

hours after application.

A comparison of systemic and intracutaneous absorption for Preparation II (Figures 6 and 7), where dimethyl sulfoxide was the vehicle, shows that perhaps there is an indirect relationship between the systemic and the intracutaneous absorption of hydrocortisone from this preparation during the initial stages of absorption. Between the two and four hour rest periods there is a sharp increase in plasma activity corresponding to a rapid decrease in dermal hydrocortisone. This may be a result of the dimethyl sulfoxide altering the barrier function of the stratum corneum thereby allowing relatively large amounts of hydrocortisone into the dermis. This, in turn, should be followed by the establishment of a high hydrocortisone concentration gradient between the dermis and the circulatory system which would probably result in a high clearance rate of hydrocortisone from the dermis into the systemic circulation. From four to 16 hours there is a gradual decrease in tissue levels of hydrocortisone. Due to the abrupt change in dermal levels of hydrocortisone after four hours, the amount of drug passing into the systemic circulation would naturally decrease. This can be confirmed by the much slower increase in plasma activity between four and eight hours followed by a decrease in activity after 16 hours. The pattern of systemic absorption from Preparation II is definitely not proportional to intracutaneous absorption and therefore

intracutaneous measurements should not be substituted for by systemic measurements.

As well as systemic absorption not being proportional to intracutaneous absorption, systemic measurements do not enable the early detection of absorption. Relatively large standard deviations have been obtained in the systemic measurement used in the present study (Tables III and IV) which indicates a lack of precision in this method as compared to intracutaneous measurements. Also, plasma is not recognized as an accurate measure of systemic absorption, since as the rest time increases the amount of drug stored in various tissues, metabolized and excreted increases along with the amount of drug being absorbed. For these reasons and since absorption into the dermal tissue is of prime interest in the present study systemic measurements were not made in future experiments.

In summary it would appear from the results of Experiments I and II that the intracutaneous absorption of topically applied hydrocortisone can be measured satisfactorily by the method employed, that the effect of dimethyl sulfoxide on such absorption appears to be significant and that the intracutaneous measurement approach probably serves as a more sensitive method of analysis than does the systemic measurement for the evaluation of the effect of dimethyl sulfoxide on hydrocortisone absorption.

B. INTRACUTANEOUS ABSORPTION OF HYDROCORTISONE AS A
FUNCTION OF DIMETHYL SULFOXIDE CONCENTRATION AND
REST TIME

Upon application to the human forearm, Preparation II caused an irritating, burning sensation. Consequently, this preparation probably would not be therapeutically acceptable. Therefore, further investigations were carried out. They were directed at studying the effects of lesser concentrations of dimethyl sulfoxide in a cream base on the intracutaneous absorption of hydrocortisone. The primary objective in this respect was to see if such concentrations of dimethyl sulfoxide which may be therapeutically acceptable would still influence the intracutaneous absorption of this glucocorticoid. The basic formulations used in the following experiments were virtually the same as Preparation I since relatively large amounts of dimethyl sulfoxide could be substituted for water in this base. Preparations containing 50, 25 and 12.5 per cent dimethyl sulfoxide were prepared on this basis (see p. 45).

Experiment III was performed using 50 per cent dimethyl sulfoxide in the cream preparation (Preparation III). The results of this experiment are shown in Table V. Fifty per cent dimethyl sulfoxide produced a much greater intracutaneous absorption of hydrocortisone than did zero per cent at all rest times studied. After two hours the 50 per cent dimethyl sulfoxide preparation gave dermal levels of hydrocortisone which were nearly as high

TABLE V
INTRACUTANEOUS ABSORPTION OF ³H-LABELED
HYDROCORTISONE FROM A 50% DIMETHYL
SULFOXIDE PREPARATION¹

REPLICATES	ABSORPTION ² (CORRECTED COUNTS PER MINUTE)			
	REST TIME (HOURS)			
	2	4	8	16
1	104,549	72,769	88,399	14,954
2	97,394	81,648	70,594	30,049
3	115,773	109,688	45,603	9,636
4	114,669	84,627	55,544	15,577
5	95,620	102,506	81,680	11,434
6	148,540	91,302	72,497	20,241
Average	112,758	90,590	69,053	16,982
Standard Deviation	+19,433	+13,894	+16,022	+7,378

¹ One per cent concentration, 1 mc/Gm specific activity (corrected) hydrocortisone in Aqueous Cream base - 50 per cent dimethyl sulfoxide.

² Activity of absorbed hydrocortisone in guinea pig dermal tissue.

as those obtained when 100 per cent dimethyl sulfoxide was used as the vehicle. Previous investigators (100, 110, 112) have suggested that relatively large concentrations of dimethyl sulfoxide, approximately 70 per cent or more, were required to cause any significant change in the absorption of other molecules. Results of intracutaneous absorption in this experiment indicate that such is not necessarily the case with hydrocortisone since 50 per cent dimethyl sulfoxide appears to be almost as effective as does 100 per cent dimethyl sulfoxide in enhancing such absorption after two hours. Fifty per cent dimethyl sulfoxide in a cream base also has the advantage of not causing the burning sensation observed with 100 per cent dimethyl sulfoxide. Therefore, it would be more acceptable to the patient. The results also indicate that although the initial tissue levels of hydrocortisone are relatively high they progressively decrease as the rest period is increased from two to 16 hours.

The effect of 25 per cent dimethyl sulfoxide (Preparation IV) on the intracutaneous absorption of hydrocortisone was determined in Experiment IV, the results of which are presented in Table VI. Twenty-five per cent dimethyl sulfoxide also causes a significant increase in dermal absorption over the zero per cent dimethyl sulfoxide preparation. As with the 50 per cent preparation the hydrocortisone in the dermis decreases

TABLE VI
INTRACUTANEOUS ABSORPTION OF ^3H -LABELED
HYDROCORTISONE FROM A 25% DIMETHYL
SULFOXIDE PREPARATION¹

REPLICATES	ABSORPTION ² (CORRECTED COUNTS PER MINUTE)			
	REST TIME (HOURS)			
	2	4	8	16
1	55,751	30,253	12,207	13,803
2	44,555	33,027	24,023	8,834
3	26,945	35,460	25,375	11,152
4	20,745	23,897	10,761	17,263
5	36,378	39,416	15,472	17,517
6	28,257	15,868	23,460	11,162
Average	35,439	29,654	18,533	13,289
Standard Deviation	$\pm 12,932$	$\pm 8,531$	$\pm 6,553$	$\pm 3,546$

¹ One per cent concentration, 1 mc/Gm specific activity (corrected) hydrocortisone in Aqueous Cream base - 25 per cent dimethyl sulfoxide.

² Activity of absorbed hydrocortisone in guinea pig dermal tissue.

as the rest time increases although the rate of decrease due to the 25 per cent dimethyl sulfoxide does not appear to be as rapid as with the 50 per cent preparation.

In Experiment V 12.5 per cent dimethyl sulfoxide (Preparation V) was used to see if a relatively low concentration of dimethyl sulfoxide had any significant effect on the intracutaneous absorption of hydrocortisone. As in Experiments III and IV the 12.5 per cent preparation (Table VII) gave a significant increase in dermal hydrocortisone over the zero per cent dimethyl sulfoxide preparation. The rate of decrease of hydrocortisone levels in the dermis appeared to be less than that observed with 25 per cent dimethyl sulfoxide.

The amount of hydrocortisone in the dermis at each rest time due to the various concentrations of dimethyl sulfoxide was compared to the zero per cent dimethyl sulfoxide preparation by using the Student's "t" test (174). After two, four, eight and 16 hour rest periods the amount of hydrocortisone in the dermal tissue due to Preparations II, III, IV and V was significantly greater than the amount of absorption resulting from the application of the zero per cent dimethyl sulfoxide preparation.

RELATIVE EFFECTS OF DIMETHYL SULFOXIDE CONCENTRATION AND REST TIME ON HYDROCORTISONE ABSORPTION

To produce a relative comparison of the effect of dimethyl sulfoxide concentration and of rest time on the

TABLE VII
INTRACUTANEOUS ABSORPTION OF ^3H -LABELED
HYDROCORTISONE FROM A 12.5% DIMETHYL
SULFOXIDE PREPARATION¹

REPLICATES	ABSORPTION ² (CORRECTED COUNTS PER MINUTE)			
	REST TIME (HOURS)			
	2	4	8	16
1	19,516	10,708	6,277	4,194
2	18,422	11,827	6,531	8,940
3	16,199	12,766	14,974	4,022
4	15,438	19,602	14,973	3,412
5	14,234	8,499	6,004	8,057
6	14,067	14,548	5,994	8,117
Average	16,313	12,992	9,125	6,124
Standard Deviation	$\pm 2,229$	$\pm 3,818$	$\pm 4,534$	$\pm 2,495$

¹ One per cent concentration, 1 mc/Gm specific activity (corrected) hydrocortisone in Aqueous Cream base - 12.5 per cent dimethyl sulfoxide.

² Activity of absorbed hydrocortisone in guinea pig dermal tissue.

intracutaneous absorption of hydrocortisone the average absorption values from Tables I, II, V, VI and VII are presented graphically in Figure 8. The magnitude of increase in absorption due to each concentration or time variable is compared to a factor of one which arbitrarily represents the degree of absorption of the zero per cent dimethyl sulfoxide preparation at the two hour rest time.

EFFECT OF DIMETHYL SULFOXIDE CONCENTRATION

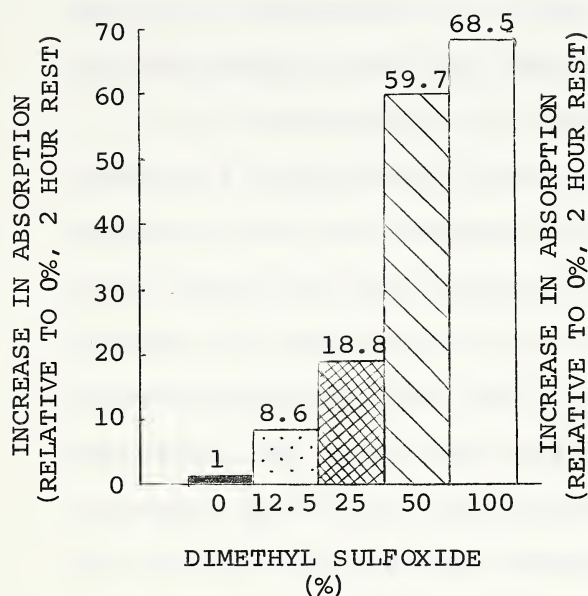
The dermal levels of hydrocortisone at the end of the two hour rest period increase as the concentration of dimethyl sulfoxide increased from zero to 100 per cent (Figure 8 (a)). All hydrocortisone levels were considerably greater than those which resulted from the zero per cent dimethyl sulfoxide preparation. The 100 per cent and 50 per cent preparations resulted in 68.5 and 59.7 fold increases respectively over the zero per cent dimethyl sulfoxide preparation. The 25 per cent preparation produced an 18.8 fold increase while the 12.5 per cent formulation caused an 8.6 fold increase.

After four hours all of the dimethyl sulfoxide preparations again showed a significantly higher dermal level of hydrocortisone than resulted from the zero per cent dimethyl sulfoxide preparation (Figure 8 (b)). The increase was not as great however as that observed after two hours. The 12.5, 25, 50 and 100 per cent dimethyl sulfoxide preparations produced 6.9, 15.7, 47.9 and 21.6

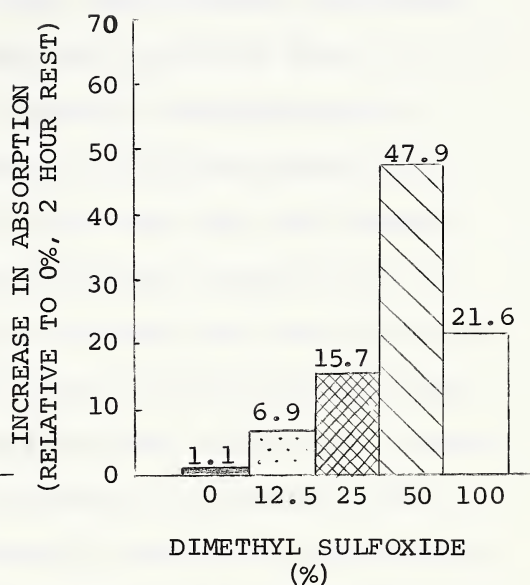
Figure 8: Relative increases* in the intracutaneous absorption of ^3H -labeled hydrocortisone due to different concentrations of dimethyl sulfoxide at various rest times.

* Relative increases compared to absorption from 0% preparation at two hours which arbitrarily has been assigned a factor of 1.

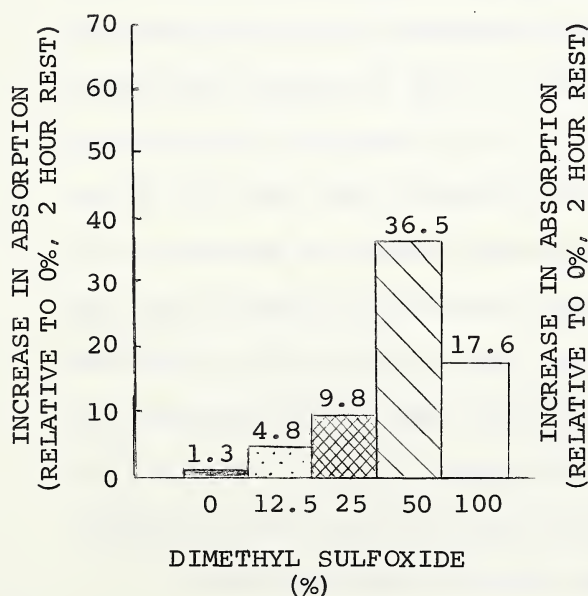
(a) 2 HOUR REST TIME



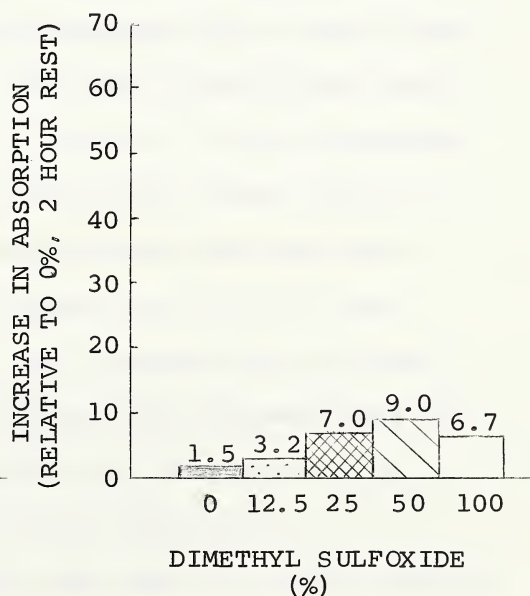
(b) 4 HOUR REST TIME



(c) 8 HOUR REST TIME



(d) 16 HOUR REST TIME



fold increases respectively over the zero per cent - two hour rest combination. It is interesting to note here that the increase due to 100 per cent dimethyl sulfoxide is considerably less than that due to 50 per cent.

All concentrations of dimethyl sulfoxide again produced a significant increase in hydrocortisone absorption over that produced by the zero per cent preparation after the eight hour rest period (Figure 8 (c)) although the magnitude of this increase was less than the increase observed after four hours. As in the four hour experiment the 50 per cent preparation caused the largest increase, 36.5 fold. This increase is followed by the 100, 25 and 12.5 per cent dimethyl sulfoxide preparations respectively which, in turn, produced 17.6, 9.8 and 4.8 fold increases.

The magnitude of the effect of dimethyl sulfoxide on absorption was considerably reduced after the 16 hour rest time (Figure 8 (d)). The 100, 50 and 25 per cent preparations resulted in 6.7, 9.0 and 7.0 fold increases while 12.5 per cent dimethyl sulfoxide caused only a 3.2 fold increase in dermal hydrocortisone over the zero per cent dimethyl sulfoxide preparation at two hours. Absorption from the 100 per cent preparation continued to decrease faster than that from other preparations and in the above analysis appeared to produce less of an increase than did the 25 per cent formulation.

No mathematical constant was apparent which equated

the degree of intracutaneous hydrocortisone absorption with the concentration of dimethyl sulfoxide in the formulation. The exercise to derive such a factor would become purely academic since the absorption data from guinea pig skin could not be readily adapted for projecting formulations for human use. Some general observations, however, can be made from Figure 8. First of all, dimethyl sulfoxide had a significant effect on the dermal absorption of hydrocortisone even at the minimum concentration of 12.5 per cent. Since this formulation caused no qualitative discomfort upon application to humans its consideration as an adjuvant for topical dermal therapy becomes optimistic. Furthermore, the potential of dimethyl sulfoxide in this respect is increased by the fact that it exerts its effect as early as two hours after application. It must be remembered, however, that the increase in availability of hydrocortisone in the dermal tissues due to the influence of dimethyl sulfoxide does not necessarily mean better therapy. For example, the mechanism of hydrocortisone action in the therapy of inflammation may be interfered with by dimethyl sulfoxide.

EFFECT OF REST TIME

The following comparisons again are based on the zero per cent dimethyl sulfoxide preparation, two hour rest time which has been assigned a factor of one. Figure 8 shows that the absorption of hydrocortisone from the zero

per cent dimethyl sulfoxide preparation increased with time although the increase from two to 16 hours was only 1.5 fold. On the otherhand, dermal hydrocortisone levels from all of the dimethyl sulfoxide preparations decreased with time. Furthermore, the rate of decrease appears to increase as the concentration of dimethyl sulfoxide increases. For example, while dermal hydrocortisone levels with the 12.5 per cent dimethyl sulfoxide preparation decreased from two to 16 hours by factors of 8.6 to 3.2, dermal levels from 100 per cent dimethyl sulfoxide decreased over the same time periods by factors of 68.5 to 6.7.

Whether the rates of dermal depletion of hydrocortisone absorbed from these various preparations are influenced by the potential of concentration gradients between the dermis and underlying vascular beds or by some mechanism of dimethyl sulfoxide cannot be concluded from the results of the present investigation.

The reason for a negative absorption gradient as a function of time for the dimethyl sulfoxide preparations as opposed to a positive gradient for hydrocortisone without dimethyl sulfoxide may be postulated, however, by considering a three compartment system made up of the stratum corneum, the dermis and the vascular system. The slow release of hydrocortisone without dimethyl sulfoxide from the stratum corneum to the dermis results in this second phase not reaching a steady state of diffusion

through it at the times studied. This is supported by Table III where systemic measurements indicated little or no drug passed from the dermal phase to the vascular phase before eight hours. Consequently, at the times studied, drug is being built up in the dermis or second phase resulting in increasing dermal levels of hydrocortisone with time. On the otherhand, assuming that a steady state is reached in the dermis before two hours with the dimethyl sulfoxide preparations, the rate of drug leaving this phase to the vascular system is greater than the amount of drug entering the dermal phase from the stratum corneum. On this basis the greater the amount of drug in the dermis, the greater will be the concentration gradient between the dermis and vascular system and therefore the faster will be the depletion rate. This appeared to account for the increased rate of dermal depletion as the concentration of dimethyl sulfoxide increased.

C. EFFECT OF DIMETHYL SULFOXIDE ON RATE OF RELEASE OF HYDROCORTISONE FROM VEHICLES

Although the primary mechanism by which dimethyl sulfoxide increases the intracutaneous absorption of hydrocortisone is expected to occur in vivo through the reduction of the skin's main barrier, the possibility of an in vitro effect cannot be eliminated. It has been shown that the rate of release of a drug by the vehicle to the surface of the skin can influence the rate of

absorption (175). Therefore, the incorporation of increasing concentrations of dimethyl sulfoxide in the preparations used in this study might influence such release by modifying either the viscosity of the preparation or the solubility of the drug in the preparation (176). Consequently, it was decided to compare the effect of dimethyl sulfoxide on the rate of release of hydrocortisone from the test preparations with the subsequent effect observed in absorption.

In Experiment VI the effect of dimethyl sulfoxide on the release of hydrocortisone from the zero, 12.5, 25 and 50 per cent dimethyl sulfoxide creams was determined. It was decided to use two and 16 hour diffusion periods since the maximum and minimum degrees of intracutaneous absorption were respectively shown at these times. This effect was studied by determining the per cent of hydrocortisone which diffused out of each vehicle through a membrane and into another semi-solid medium (see p. 61).

Table VIII gives a relative comparison of the effect of dimethyl sulfoxide on the release of hydrocortisone and of the amount of hydrocortisone in the dermis after two hours from each cream tested. As is shown, the in vitro increase in release due to dimethyl sulfoxide over the zero per cent preparation at all three concentrations was very low when compared to the substantial increases in intracutaneous hydrocortisone.

TABLE VIII

IN VITRO RELEASE¹ AS COMPARED TO THE IN VIVO
INTRACUTANEOUS ABSORPTION² OF ³H-LABELED
HYDROCORTISONE AFTER TWO HOURS

CONCENTRATION DIMETHYL SULFOXIDE (%)	<u>IN VITRO</u>		<u>IN VIVO</u>	
	% DIFFUSED	INCREASE ³ OVER 0%	ABSORPTION (CORRECTED COUNTS PER MINUTE)	INCREASE ³ OVER 0%
0	0.03726	1.00X	1,890	1.00X
12.5	0.04572	1.23X	16,313	8.63X
25	0.05040	1.35X	35,439	18.75X
50	0.12480	3.35X	112,758	59.66X

¹ Release of ³H-labeled hydrocortisone across a cellulose membrane in an in vitro cell.

² Absorption of ³H-labeled hydrocortisone by guinea pig dermal tissue.

³ Factor increase over value obtained for 0% dimethyl sulfoxide.

By 16 hours the increase in release of hydrocortisone due to 12.5, 25 and 50 per cent dimethyl sulfoxide as compared to the zero per cent preparation had changed very little from the increases observed after two hours (Table IX). However, the increase in intracutaneous levels of hydrocortisone from the dimethyl sulfoxide preparations over the zero per cent dimethyl sulfoxide preparation was substantially reduced by 16 hours. The increases, however, were still significantly greater than the in vitro increases in release at this time.

From these results it seems reasonable to assume that the increases in hydrocortisone release from the vehicles due to dimethyl sulfoxide do not in themselves account for the dramatic subsequent intracutaneous absorption. The literature suggests that the primary mechanism of dimethyl sulfoxide appears to occur in vivo through a physical alteration of the skin barrier to penetration. The results of the present investigation also support an in vivo mechanism of dimethyl sulfoxide to promote the major increase in intracutaneous absorption.

TABLE IX

IN VITRO RELEASE¹ AS COMPARED TO THE IN VIVO
INTRACUTANEOUS ABSORPTION² OF ³H-LABELED
HYDROCORTISONE AFTER SIXTEEN HOURS

CONCENTRATION DIMETHYL SULFOXIDE (%)	<u>IN VITRO</u>		<u>IN VIVO</u>	
	% DIFFUSED	INCREASE ³ OVER 0%	ABSORPTION CORRECTED (COUNTS PER MINUTE)	INCREASE ³ OVER 0%
0	0.13133	1.00X	2,815	1.00X
12.5	0.21060	1.60X	6,124	2.18X
25	0.28399	2.16X	13,289	4.72X
50	0.33289	2.53X	16,982	6.03X

¹ Release of ³H-labeled hydrocortisone across a cellulose membrane in an in vitro cell.

² Absorption of ³H-labeled hydrocortisone by guinea pig dermal tissue.

³ Factor increase over value obtained for 0% dimethyl sulfoxide.

SUMMARY AND CONCLUSIONS

1. The quantitative effect of dimethyl sulfoxide on the transepidermal, intracutaneous absorption of hydrocortisone has been measured using guinea pig skin.
2. The intracutaneous absorption of hydrocortisone was detected as early as two hours after its topical application with and without dimethyl sulfoxide.
3. Concentrations of dimethyl sulfoxide ranging from 12.5 to 100 per cent caused significant increases in the intracutaneous absorption of hydrocortisone at each of several rest times studied.
4. The major effect of dimethyl sulfoxide on the intracutaneous absorption of hydrocortisone appeared to occur within two hours after topical application.
5. The levels of hydrocortisone in the dermis decreased with increasing rest times in the presence of dimethyl sulfoxide and the rate of decrease appeared to accelerate as the concentration of dimethyl sulfoxide was increased.
6. The primary mechanism by which dimethyl sulfoxide enhances the intracutaneous absorption of hydrocortisone appeared to be by a direct effect on the skin barrier.

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